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SLOVENSKI VETERINARSKI ZBORNIK





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SLOVENIAN VETERINARY RESEARCH

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Slov Vet Res 2008; 45 (4)

Original Research Papers	
Kataria N, Kataria AK, Gahlot AK. Use of plasma gastrin and pepsinogen levels as diagnostic markers of abomasal dysfunction in Marwari sheep of arid tract	21
Kataria N, Kataria AK, Gahlot AK. Ambient temperature associated variations in serum	
hormones and interrelated analytes of broiler chikens in arid tract	27
Case Report	
Gombač M, Švara T, Zdovc I, Juntes P, Pogačnik M. Yersiniosis in captive capybaras	
(Hydrochaeris hydrochaeris)	35
On the occasion of 200 th anniversary of the birth of Dr Janez Bleiweis	
Editorial (Majdič G) 14	41
Summaries of Supplement 12 (Bavdek SV, Vodopivec P, Burnik J, Pengov A, Jurca J, Osterc J)	42
Subject Index Volume 45, 2008	47
Author Index Volume 45, 2008	49

USE OF PLASMA GASTRIN AND PEPSINOGEN LEVELS AS DIAGNOSTIC MARKERS OF ABOMASAL DYSFUNCTION IN MARWARI SHEEP OF ARID TRACT

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Summary: Hormone gastrin is secreted from gastrin cells of pyloric region of abomasum into the blood circulation, thence to reach the parietal cells and is an important stimulator of acid and pepsinogen secretion. Pepsinogen, a proenzyme is an inactive form of pepsin which is the most important proteolytic enzyme of gastric juice. To assess the role of gastrin and pepsinogen in the diagnosis of abomasal parasitism or disorders, the gastrin and pepsinogen levels were determined in the plasma of Marwari breed of sheep belonging to farmers' stock of arid tract of Rajasthan state, India. The animals, from which the blood samples collected, were grouped into healthy, haemonchus infected and drought affected. In healthy animals sampling was carried as one time random sampling and three times sampling. The overall mean values of plasma gastrin and pepsinogen in healthy Marwari sheep were 103.45± 10. 41 pg/ml and 153.61± 13.21 mU tyrosine, respectively. In haemonchus infected and drought affected sheep a significant (p≤0.05) increase was observed in the mean values for both the parameters in comparison to that of healthy stock. The highest values for both the parameters were observed in haemonchus infected animals. The sampling time did not affect the gastrin and pepsinogen levels. This showed that feeding did not affect the levels of gastrin and pepsinogen. The responses of gastrin and pepsinogen in affected sheep indicated that they can be used as diagnostic markers in animals affected with abomasal dysfunctions. Probably the presence of parasites or inanimate objects in pica damaged the mucosa causing an impairment of abomasal function. Damage to mucosa is related with higher gastrin and pepsinogen release. Further the results suggested that one time random sampling can be carried out in suspected clinical cases for the determination of plasma gastrin and pepsinogen. The data obtained can be used as a base line for the future studies in this direction in Marwari sheep or other breeds of sheep.

Key words: drought; gastrin; haemonchosis; Marwari sheep; pepsinogen; pica

Introduction

Hormone gastrin is secreted from gastrin cells of pyloric region of abomasum into the blood circulation, thence to reach the parietal cells and is an important stimulator of acid and pepsinogen secretion (1). Pepsinogen, a proenzyme is an inactive form of pepsin which is the most important proteolytic enzyme of gastric juice. In the gastric or abomasal lumen pepsinogen is converted into pepsin in the

Received: 30 April 2008 Accepted for publication: 17 October 2008 presence of acid (1, 2). In ruminant blood a certain physiological level of pepsinogen exists (3). Blood levels of pepsinogen can be used in the diagnosis of abomasal parasitism or disorders (4). The increased plasma levels of pepsinogen are caused due to its leakage into blood vessels from damaged abomasal mucosa (5). Increased activation of pepsinogen into pepsin by enhanced acidity of gastric contents can cause ulcers in humans and animals (6).

There are reports, although scant, on the use of plasma pepsinogen and gastrin as indicators of gastric dysfunctions in man (7, 8) and animals (4, 9, 10). However, there is no literature available on this as-

pect in Marwari breed of sheep. Also, there is rarity of literature regarding normal values of pepsinogen and gastrin in animals.

The Marwari sheep is an important breed of sheep in arid regions and frequently face problems of droughts (11). During drought periods animals feed on non-conventional feeds and develop pica in which condition they start eating in-animate objects (2). The gastrointestinal worm infestation is also a great problem in sheep. In these circumstances mucosa of abomasum is damaged greatly impairing digestive functions as proper gastric mucosa plays an important role in digestive processes. However, many a times damage to abomasal mucosa remains undiagnosed. The determination of plasma levels of gastrin and pepsinogen can be an important aid for taking timely measures to treat such animals (2, 5, 6). The present investigation was planned to explore the possibilities of role of plasma gastrin and pepsingen as diagnostic tools in assessing abomasal involvement in Marwari sheep and to set the normal values of these parameters. In the field conditions to overcome the difficulty of repeated sampling, the investigation was also aimed to collect samples at different times to find out the effect, if any, of different sampling time on the levels of gastrin and pepsinogen.

Material and methods

Plasma gastrin and pepsinogen levels were determined in sheep of Marwari breed belonging to farmers' stock of arid tract of Rajasthan state, India. The animals were maintained on the free-range grazing system. The animals, from which the blood samples collected, were grouped into healthy, haemonchus infected and drought affected. In healthy animals sampling was carried out in two patterns i.e. one time random sampling and three times sampling. In one time random sampling cases, the blood was drawn once, irrespective of time, for the preparation of plasma from 17 apparently healthy adult Marwari sheep of either sex (10 male and 7 female). The samples were collected as a part of routine health checkup during moderate ambience (maximum temperature varied between 26 and 29 °C) and animals were free from endo-parasites as assessed by routine faecal examination. In three times sampling cases blood was obtained from the same 17 adult Marwari sheep by samplings three times i.e. morning, mid-day and evening and the means were presented irrespective of sex.

In the haemonchus infected group, blood samples were obtained (from the slaughter house) from other 18 Marwari sheep of either sex (9 male and 9 female) at the time of slaughtering which had haemonchus infection detected at the time of slaughter.

In the drought affected group, 20 blood samples (10 male and 10 female) were collected from drought affected Marwari sheep of arid tract having the history of pica belonging to farmers' stock.

All the samples were collected in sterile tubes containing EDTA (di-potassium) as an anticoagulant for plasma separation. Plasma gastrin levels were determined by double antibody gastrin 125, radioimmunoassay (RIA Kit, DPC, USA) as per the manual supplied with RIA kit in the Radio Isotope Laboratory, College of Veterinary and Animal Science, Bikaner, India. The materials supplied in the kit included gastrin antiserum, 125, gastrin, gastrin calibrators and precipitating solution consisting of goat anti-rabbit gamma globulin and dilute polyethylene glycol in saline. In the double antibody gastrin procedure a competition was there between 125, labeled gastrin and gastrin hormone in plasma for the sites on gastrin specific antibody. After incubation for 2 hours (15-28°C), bound was separated from free by PEG accelerated double antibody method. A foam decantation rack was used to decant the supernatant, retaining the precipitate for counting. Counting was carried out by using 125, Gamma counter (EC, India).

Plasma pepsinogen was determined by a colorimetric method (7) with modifications (12) using bovine albumin fraction V (CDH, India) and glycine (Loba Chemie, India) to prepare substrate mixture. In the method plasma was added to a substrate mixture (0.9 % bovine albumin fraction V in glycine buffer, pH 2.0) and mixed thoroughly. An aliquot of this mixture was incubated in a water bath at 37°C for 24 hours. Then reaction was stopped by adding 4 % trichloroacetic acid (Glaxo, India) solution (12).After filtration of the protein precipitate the concentration of acid soluble tyrosine was determined by Folin Coicalteau's reagent (CDH, India). For this in each 2 ml of filtrate 0.5 ml of Folin Coicalteau's reagent and 10 ml of 0.25N sodium hydroxide (Qualigens, India) were added. Two ml of tyrosine (BDH, India) standard was also treated in the same way. The optical density was read at 680 mµ wave length using a spectrophotometer (Systronics, India). The enzyme activity was expressed as milli-units (mU) tyrosine (13).

Correlation was determined by MSTAT computer programme. Statistical significance for individual

parameter between male and female, healthy and infected, healthy and drought affected, morning and mid-day sampling, and morning and evening sampling was analysed by paired- t test (14).

Results

The mean values of plasma gastrin and pepsinogen in Marwari sheep are presented in two tables. Mean values that are presented in the table 1 belong to healthy, haemonchus infected and drought affected animals. They are further grouped into male and female animals. Non significant (p>0.05) difference was observed in the respective mean values of plasma gastrin and pepsinogen between male and female within each category i.e. healthy, haemonchus infected and drought affected animals.

Table 1: Plasma levels of gastrin and pepsinogen inhealthy, haemonchus infected and drought affected Marwari sheep

S.No.	Groups	Gastrin	Pepsinogen
		(pg/ml)	(mU tyrosine)
1.	Healthy		
(a)	Overall (17)	103.45± 10.41	153.61± 13.21
(b)	Male (10)	100.32±11.41	150.41± 14.11
(c)	Female (7)	106.41 ± 13.21	156.4 ± 11.61
2.	Haemonchus Infected		
(a)	Overall (18)	489.61 ± 12.33 ^{bd}	772.31 ± 12.2 ^{bd}
(b)	Male (9)	$485.45 \pm 13.6 \ ^{\rm bd}$	769.21 ± 10.12 bd
(c)	Female (9)	$494.32 \pm 11.00^{\rm bd}$	$776.30 \pm 14.40 \ ^{\rm bd}$
3.	Drought affected		
(a)	Overall (20)	310.33± 15.1 ^{bd}	460.01 ± 11.5 ^{bd}
(b)	Male (10)	308.97 ± 16.0 ^{bd}	457.87 ± 12.0 ^{bd}
(c)	Female (10)	311.51 ± 14.9 ^{bd}	363.45 ± 10.3 ^{bd}

1. Figures in the parentheses indicate number of animals.

2. Healthy overall mean value of each parameter was compared with the respective mean value of infected and drought affected animals. The significant ($p\le 0.05$) variation has been shown by superscript 'b'.

3. The mean values of each parameter of haemonchus affected and drought affected animals were compared and means superscribed by letter'd' within a given parameter differed significantly (p \leq 0.05) from the respective means of other group.

The overall mean values of plasma gastrin and pepsinogen of haemonchus infected and drought affected animals were significantly ($p \le 0.05$) higher than the respective overall mean value of healthy animals. Haemonchus infected animals showed a greater rise in the mean values of both the parameters as compared to drought affected animals.

Table 2: Plasma levels of gastrin and pepsinogen in healthy
Marwari sheep

S.No.	Groups	Gastrin (pg/ml)	Pepsinogen (mU tyrosine)
A.	One time random sampling Overall (17)	103.45± 10.41	153.61± 13.21
B.	Three times sampling Overall, three times sampling (51)	104.0± 10.12 ª	152.6 ± 13.0 ª
(a)	Morning sampling (17)	99.2 ± 11.0 ac	147.5 ± 12.75 ac
(b)	Mid –day sampling (17)	105.0 ± 9.23 ac	$152.7 \pm 15.62 \ ^{\mathrm{ac}}$
(c)	Evening sampling (17)	109.8 ± 11.3 ac	$159.4\pm12.56^{\rm ac}$

1. Figures in the parentheses indicate number of observations from 17 healthy animals.

2. Overall mean value of one time random sampling of each parameter was compared with the respective mean value of overall three times sampling, morning, mid-day and evening sampling. The non-significant (p>0.05) variation has been shown by superscript 'a'.

3. The non-significant (p>0.05) variation among the mean values during morning, mid-day and evening samplings has been shown by superscript 'c'.

Mean values presented in the table 2 are of healthy animals categorised according to one time random sampling and three times sampling. Overall mean value of one time random sampling of each parameter was compared with the respective mean value of overall three times sampling, morning, mid-day and evening sampling. The non-significant (p>0.05) variation was observed in each case. Further in three time sampling category the mean values obtained during morning, mid-day and evening samplings were compared from each other and the variations were non-significant (p>0.05) among the mean values.

A significant ($p \le 0.05$) correlation ($r = 0.921^*$) between the values of plasma gastrin and pepsinogen in haemonchus infected and drought affected sheep was observed.

Discussion

The overall mean value of plasma gastrin in the present study for healthy animals was found in the middle range of the values obtained by earlier workers in sheep (15,16) and it was more or less similar to those recorded in calves (9) and cows (17). The healthy overall mean value of plasma pepsinogen in the present study was almost comparable to those given for lambs (4) and sheep (16).

The non significant (p>0.05) effect of sampling time showed that feeding did not affect the levels of gastrin and pepsinogen in present study. It could be due to the fact that in ruminants the abomasum receives a continuous, though variable inflow of forestomach material (18) therefore sampling time did not affect the values of gastrin and pepsinogen. Earlier report (15) also suggests that mean plasma gastrin concentrations do not increase in response to feeding.

The pattern of changes in the values of plasma gastrin and pepsinogen was similar in the present study. The values of both the parameters increased in haemonchus infected and drought affected animals when compared to healthy animals. Similar findings were reported in camel (2). Serum gastrin concentrations were observed elevated in parasitised sheep (19). Earlier researchers (4) found an increase in plasma gastrin and pepsinogen levels in lambs infected with haemonchiasis and in sheep infected with Ostertagia circumcincta (6, 16).

Earlier workers have reported increased levels of serum pepsinogen in cattle with abomasal ulcers (6, 20); of plasma gastrin in cattle with bleeding abomasal ulcers (17) and of serum pepsinogen and gastrin in bovine ostertagiasis (9, 12).

A significant correlation between the values of plasma gastrin and pepsinogen in haemonchus infected and drought affected sheep (4) indicated that both the parameters can be used simultaneously or individually to assess the abomasal dysfunction. Plasma pepsinogen estimations are performed routinely in many laboratories as an aid in the diagnosis of ostertagiasis. Elevated values also occur in haemonchosis and levels decline after effective anthelmintic treatment. Plasma gastrin concentrations reflect the size of abomasal worm burden in both young and older animals (21).

Haemonchus contortus is one of the most abundant infectious agents in sheep around the world, causing great economic damage to sheep farms. Nematode larvae developing within the glands cause local loss of parietal cells and mucous cell hyperplasia whereas reduced hydrochloric acid secretion, increased serum gastrin and pepsinogen concentrations and generalized histological changes are associated with parasites in the abomasal lumen. Parietal cells with dilated canaliculi and degenerative changes typical of necrosis are present soon after the transplantation of adult worms, and abomasal secretion is also affected (22). The massive doses of parasites produce a significant decrease in acidity and increase in sodium ion concentration of abomasal fluid. This is followed by an increase in plasma pepsinogen (23). The changes in abomasal environment are due to increased permeability of mucosa. Rise in abomasal pH is an important stimulus for gastrin release (2).

Anaerobic bacteria survive in greater numbers as the pH rises. Failure to lyse bacteria may affect adversely the nutrition of the host. The parasites may initiate the pathophysiology through the release of excretory or secretory products which either act directly on parietal cells or indirectly through enterochromaffin-like cells by provoking inflammation or by disrupting the protective mucosal defense system. Parietal cell dysfunction leads to loss of mature chief cells, mucous cell hyperplasia and hypergastrinaemia. Inflammation increases circulating pepsinogen concentrations and may also contribute to increased gastrin secretion (22).

The responses of gastrin and pepsinogen in affected Marwari sheep in the present study certainly indicated towards their diagnostic significance in abomasal dysfunctions. Probably the presence of parasites or inanimate objects in pica damaged the mucosa causing an impairment of abomasal function. Recent work in camel has demonstrated a marked increase in levels of the plasma gastrin and pepsinogen in pica affected camels which had sand in their third compartment damaging the mucosa (2). Damage to mucosa causes a drop in hydrochloric acid production (22) resulting in elevation of gastric pH which is accompanied by higher gastrin and pepsinogen release (16, 22, 24). Further the results suggested that one time random sampling can be carried out in suspected clinical cases for the determination of plasma gastrin and pepsinogen as reported by earlier workers (2). The data obtained can be used as a base line for the future studies in this direction in Marwari sheep or other breeds of sheep.

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UPORABA VREDNOSTI GASTRINA IN PEPSINOGENA V KRVNI PLAZMI ZA DIAGNOSTIČNE POKAZATELJE OKVARE SIRIŠČNIKA PRI OVCAH PASME MARVARI V SUŠNIH PODROČJIH

N. Kataria, A.K. Kataria, A.K. Gahlot

Povzetek: Hormon gastrin izločajo gastrinske celice v piloričnem delu siriščnika v krvni obtok. Preko krvi gastrin prihaja do parietalnih celic in pomembno vpliva na izločanje želodčne kisline in pepsinogena, ki je proencim, neaktivna oblika pepsina, najpomembnejšega proteolitičnega encima v želodčnem soku. Da bi določili vlogo gastrina in pepsinogena pri diagnostiki zajedavskih invazij in drugih motenj siriščnika, smo določili vrednosti gastrina in pepsinogena v krvni plazmi ovc pasme marvari, ki so del čred v polpuščavskem delu zvezne države Radžastan v Indiji. Živali, pri katerih je bila odvzeta kri, smo razdelili v tri skupine: zdravo, invadirano s hemonhusi in prizadeto zaradi pomanjkanja vode. Pri zdravih živalih smo vzorce krvi jemali na dva načina: z enkratnim naključnim odvzemom ali pa trikrat zapored v istem dnevu. Skupne povprečne vrednosti gastrina in pepsinogena v plazmi zdravih ovc so bile 103,45 ± 10,41 pg/ml oz. 153,61 ± 13,21 mU tirozina. Pri obeh skupinah, prizadetih bodisi zaradi zajedavcev ali suše, smo opazili statistično značilno (p £ 0,05) povečanje obeh parametrov v primerjavi z zdravimi živalmi. Čas jemanja krvi ni vplival na vrednosti gastrina in pepsinogena, kar pomeni, da tudi hranjenje ne vpliva na njune vrednosti. Ker pa se oba parametra spremenita pri prizadetih živalih, ju lahko uporabimo za diagnostična pokazatelja okvare siriščnika. Verjetno tako zajedavci kot neorganski predmeti, ki jih ovce zaužijejo med bolezenskim poželenjem po nenaravnih hrani, poškodujejo želodčno sluznico in s tem okvarijo delovanje siriščnika. Poškodba sluznice ima za posledico tudi povečano sproščanje gastrina in pepsinogena. Rezultati preiskave so tudi pokazali, da ob sumu, da gre za klinične primere, zadošča enkratni odvzem krvi ne glede na čas dneva oz. hranjenja. Pridobljeni podatki lahko služijo za osnovo pri nadalinijih proučevanjih obremenitve ovc marvari in drugih pasem.

Ključne besede: suša; gastrin; haemonchosis; ovce marvari; pepsinogen; uživanje nenaravne hrane

AMBIENT TEMPERATURE ASSOCIATED VARIATIONS IN SERUM HORMONES AND INTERRELATED ANALYTES OF BROILER CHICKENS IN ARID TRACT

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Summary: The investigation was carried out to explore the extent of changes in serum hormones and interrelated analytes of broiler chickens during varying ambient temperatures because such changes help to understand the modulations in physiological mechanisms. For this purpose sera were harvested from the broilers when the maximum temperatures were 13-16°C (low); 24-27°C (moderate) and 42-45°C (high). The values of analytes measured at moderate temperature served as control to which values of analytes measured at low and high temperatures were compared.

Higher temperatures resulted in greater variations in hormones and interrelated analytes than the lower temperatures. At 42-45°C, serum corticosterone, growth hormone, glucagon, testosterone, uric acid, creatinine, urea, glucose, cholesterol, triglycerides, free fatty acids, ASAT, ALAT, lipase and amylase increased significantly (p ≤0.05) and thyroxine, trijodothyronine, insulin, gastrin, total proteins, albumin, globulin, LDH, ICDH and pepsinogen decreased significantly (p ≤0.05) from respective control mean values. At low temperatures (13-16°C) serum T₄, T₃, LDH and ICDH were significantly (p ≤0.05) higher; glucose, cholesterol, triglycerides and free fatty acids were significantly ($p \le 0.05$) lower and corticosterone, growth hormone, insulin, glucagon, gastrin, testosterone, total proteins, albumin, globulin, uric acid, creatinine, urea, ASAT, ALAT, pepsinogen, lipase and amylase showed non significant (p>0.05) changes from respective control values. Serum prolactin, aldosterone, sodium, chloride, calcitonin, alkaline phosphatase, 5' nucleotidase and gamma glutamyl transferase were significantly (p <0.05) higher and potassium, C-PTH, calcium, phosphorus and magnesium were significantly (p <0.05) lower at 42-45°C temperatures than respective control mean values. At 13-16°C temperature serum aldosterone, sodium, chloride, alkaline phosphatase and 5' nucleotidase showed significant (p ≤0.05) fall while serum potassium marked significant ($p \le 0.05$) rise as compared to respective control mean values. It was concluded that changes in the ambient temperatures affected the glucose, fat, protein, calcium and sodium metabolisms in the way which helped the birds to survive during adverse conditions. Pattern of changes of interrelated analytes showed the physiological state of the body being governed by endocrine system.

Key words : ambient temperatures; analytes; broilers; hormones; sera

Introduction

Variations in ambient temperatures are too great in arid tract that many a times physiological adjustments in the birds are on the cost of production. Feed intake, feed conversion efficiency and growth rate in broilers are greatly affected due to high ambient temperatures (1, 2, 3). Thermal discomfort may result in improper expression of genetic potential in birds. Thermoregulation is mainly carried out on the expense of water and as a result water intake increases (4). Improper management results in development of slow dehydration. In arid tracts mortality in birds is observed during higher ambience.

Laboratory monitoring to find out effects of ambience can be done by evaluating concentrations of metabolites and electrolytes in blood as these parameters reflect the physiological state of the body during changed ambience (5). Heat, cold and dehydration are among the common stressors experienced by broilers. When the pressure from the stressors becomes excessive, or in case of psychological threats, new defense mechanisms are initiated, collectively referred to as stress responses. Determination of these stress responses is essential so that strategies can be made for making congenial ambience in broiler houses for healthier management.

Although many studies have been conducted to evaluate the effect of thermal environment in birds (6, 7) but still dearth of research is there to understand the modulations in physiological mechanisms which can be observed by assessing the changes taking place in controlling system of the body. Endocrine system is one of the regulating systems. Therefore variations in physiological mechanisms can be best understood by endocrine and metabolic responses of broilers which require serum profiling of analytes. This prompted the present investigation to determine serum hormones and interrelated analytes in broilers of arid tract during varying ambient temperatures under natural conditions.

Material and methods

Samples

Blood samples were collected to harvest the sera from 600 broilers (Male, White Leghorn chickens) of 8-9 weeks of age belonging to private poultry farms of arid tracts at the time of slaughter. The birds were maintained under natural conditions with standard feeding and watering practices. Samples (200 each) were collected when the range of maximum ambient temperatures were 13-16°C (low); 24-27°C (moderate) and 42-45°C (high). The clear, non-haemolysed sera were separated from blood samples and stored in a deep freeze at -20°C till analysis.

Analysis

To achieve the goal of the study the hormones were determined in 25 randomly selected serum samples each from low, moderate and high ambient temperatures and interrelated analytes were determined in all 600 collected serum samples. The hormones were corticosterone, thyroxine (T_4), triiodothyronine (T_3), prolactin, aldosterone, carboxy-terminal parathyroid hormone (C-PTH), calcitonin, growth hormone,

insulin, glucagons, gastrin and testosterone. The serum corticosterone was determined by fluorometric method (8) using a photofluorometer (Systronics, India). Commercial 125₁ Radio Immuno Assay (RIA) Kits were used to determine other hormones viz. thyroxine (RADIM), triiodothyronine (RADIM), prolactin(DPC), aldosterone(DPC), carboxy-terminal parathyroid hormone (DiaSorin), calcitonin (DPC), growth hormone(DPC), insulin(DPC), glucagons (DPC), gastrin (DPC) and testosterone (DiaSorin) in Radio Isotope Laboratory, College of Veterinary and Animal Science, Bikaner,India. 125₁ Gamma counter (EC, India) was used for counting radioactivity.

Interrelated analytes included metabolites, electrolytes and enzymes. Metabolites analysed were total proteins, albumin, globulin, uric acid, creatinine, urea, glucose, cholesterol, triglycerides; electrolytes were sodium, potassium, chloride, calcium and phosphorus; and enzymes were lactic dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AKP), 5'-nucleotidase, gamma glutamyl transferase (γ -GT), pepsinogen, lipase and amylase.

Serum isocitrate dehydrogenase, 5'-nucleotidase, pepsinogen, and amylase were determined by the standard spectrophotometric methods (9) by using spectrophotometer (Systronic, India). Serum lipase and free fatty acids were determined by standard titrimetric method (9). Sodium and potassium were determined by using flame photometer of Systronics, India (8) and magnesium by titan yellow method (8). Standard commercial spectrophotometric kits (Glaxo and Wipro) were used to determine remaining analytes viz .total proteins, albumin, globulin, uric acid, creatinine, urea, glucose, cholesterol, triglycerides, chloride, calcium, phosphorus, LDH, ASAT, ALAT, AKP and y-GT as per manufacturers instructions by using spectrophotometer (Systronics, India). For serum calcium necessary corrections were made (10).

All reported values are means (\pm SEM). To assess the effect of low and high ambient temperatures on individual serum analytes the respective mean value at 24-27°C was considered as control. Significance of changes was assessed by student 't' test (11).

Results

The results are presented in table 1. and 2 and analytes are grouped according to their relationship with each other.

Analytes	Maximum ambient temperature		
	13-16°C	24-27°C	42-45°C
Corticosterone, ng/ml ^a	7.9 ± 0.76	7.3 ± 0.54	$13.1 \pm 0.78^*$
Thyroxine, ng/ml ^a	18.1±0.6*	$14.8 {\pm} 0.5$	9.9±0.4*
Triiodothyronine, ng/mlª	3.9±0.2*	$2.2{\pm}0.2$	1.4±0.3*
Growth hormone, ng/mlª	49± 1.76	52 ± 2.1	$69\pm2.8^{*}$
Insulin, ng/mlª	$2.71 {\pm} 0.27$	$2.56{\pm}0.20$	1.56 ± 0.41 *
Glucagon, ng/mlª	1.66 ± 0.31	$1.71 {\pm} 0.25$	3.1±0.32*
Gastrin, pg/ml ^a	98±9.7	100±7.9	71±6.9*
Testosterone, ng/ml ^a	$1.2{\pm}0.08$	$1.4{\pm}0.07$	2.3±0.06*
Total proteins, g/L ^b	42.0 ± 3.0	45.0 ± 2.0	32.0±2.0*
Albumin, g/L ^b	21.0±3.0	$22.0{\pm}2.0$	$18.0 \pm 1.0^{*}$
Globulin, g/L ^b	21.0±3.0	$22.0{\pm}2.0$	14.0±2.0*
Uric acid, mmol/L ^b	0.312 ± 0.02	0.295 ± 0.025	$0.383 \pm 0.01*$
Creatinine, µmol/L ^b	88.4 ±0.318	79.56 ± 0.31	167.0 ±0.31*
Urea, mmol/L ^b	$0.649 {\pm}~0.05$	$0.599 {\pm}~0.02$	$0.865 \pm 0.025^{*}$
Glucose, mmol/L ^b	8.17 ±0.43*	$9.99{\pm}0.39$	$11.92 \pm 0.44*$
Cholesterol, mmol/L ^b	$3.26 \pm 0.17*$	3.88 ± 0.15	$4.22 \pm 0.20^*$
Triglycerides, mmol/L ^b	4.51±0.11*	$5.29{\pm}0.12$	5.75±0.10*
Free fatty acids, mmol/L ^b	10.98±0.34*	$12.62 {\pm} 0.30$	15.07±0.39*
Lactate dehydrogenase, IU/l ^b	1324.8±88*	1000.5 ± 87	811.7 ±78*
Isocitrate dehydrogenase, IU/lb	$13.3 \pm 1.0^{*}$	9.3 ± 0.87	7.13 ±0.94*
Aspartate amino transferase, IU/l ^b	238 ± 7.9	246 ± 10.0	354±9.87*
Alanine amino transferase, IU/l ^b	6.1±0.53	$6.9{\pm}0.56$	10.12±0.8*
Pepsinogen, U/L ^b	$2.4{\pm}0.1$	$2.51{\pm}0.4$	$1.2{\pm}0.3{*}$
Lipase, U/L ^b	$485{\pm}13.8$	511 ± 14.9	$600 \pm 15.2^*$
Amylase, U/L ^b	500±19.12	523 ± 18.21	556±17.91*

Table 1: Serum hormones, metabolites and enzymes in the broilers (Mean ± SEM)

Superscript 'a' = number of broilers at each ambience was 25

Superscript 'b' = number of broilers in each ambience was 200

*significant (p≤0.05) difference in comparison with respective control mean value

Mean values of each analyte at low (13-16°C) and high temperatures (42-45°C) were compared to respective values at control temperature 24-27°C.

At 42-45°C, serum corticosterone, growth hormone, glucagons, testosterone, uric acid, creatinine, urea, glucose, cholesterol, triglycerides, free fatty acids, ASAT, ALAT, lipase and amylase increased significantly (p ≤ 0.05) and thyroxine, triiodothyronine, insulin, gastrin, total proteins, albumin, globulin, LDH, ICDH and pepsinogen decreased significantly (p ≤ 0.05) in comparison with respective control mean values.

At low temperatures (13-16°C) serum T4, T3, LDH and ICDH were significantly ($p \le 0.05$) higher; glucose, cholesterol, triglycerides and free fatty acids were significantly ($p \le 0.05$) lower and corticosterone, growth hormone, insulin, glucagons, gastrin, testosterone, total proteins, albumin, globulin, uric acid, creatinine, urea, ASAT, ALAT, pepsinogen, lipase and amylase showed non significant (p>0.05) changes in comparison with respective control mean values.

Serum prolactin, aldosterone, sodium, chloride, calcitonin, alkaline phosphatase, 5' nucleotidase and γ -GT were significantly (p ≤ 0.05) higher and potassium, C-PTH, calcium, phosphorus and magnesium were significantly (p ≤ 0.05) lower at 42-45°C temperatures than respective control mean values. At 13-16°C temperature serum aldosterone, sodium, chloride, alkaline phosphatase and 5' nucleotidase showed significant (p ≤ 0.05) fall while serum potassium marked significant (p ≤ 0.05) rise as compared to respective control mean values. Other analytes of table 2 showed non significant (p>0.05) differences from respective control values.

Analytes	Maximum ambient temperature		
	13-16°C	24-27°C	42-45°C
Prolactin, ng/ml ^a	$56.3{\pm}4.32$	$60.9 {\pm} 4.41$	84.0±5.53*
Aldosterone, pg/ml ^a	$44.2 \pm 5.0^{*}$	$59.1 {\pm} 4.3$	99.8±8.1*
Sodium, mmol/L ^b	120.4±3.8*	$134.4{\pm}4.5$	149.9±3.2*
Potassium, mmol/L ^b	6.9±0.22*	$6.1 {\pm} 0.25$	5.1±0.3*
Chloride, mmol/L ^b	90.2±4.8*	105.6 ± 5.3	120.6±4.2*
C-PTH, ng/ml ^a	$2.4{\pm}0.2$	$2.3{\pm}0.1$	$1.9 \pm 0.1*$
Calcitonin, ng/mlª	$1.03{\pm}0.2$	$1.1{\pm}0.2$	$2.0{\pm}0.2{*}$
Calcium, mmol/L ^b	2.45 ± 0.14	2.33 ± 0.09	1.77±0.12*
Phosphorus, mmol/L ^b	1.59 ± 0.06	$1.51 {\pm} 0.09$	$1.25 \pm 0.09*$
Magnesium, mmol/L ^b	$0.86 {\pm} 0.14$	$0.82{\pm}0.08$	0.53±0.04*
Alkaline Phosphatase, U/L ^b	430.1±8.2*	$473.5{\pm}9.0$	513.2±9.3*
5'-Nucleotidase, m-U/L ^b	5.0±0.21*	7.3±0.32	15.1±1.22*
Gamma Glutamyl Transferase, U/L ^b	17.0 ± 1.3	18.23 ± 2.2	26.1±2.3*

Table 2: Serum hormones, electrolytes and enzymes in broilers (Mean \pm SEM)

Superscript 'a' = number of broilers at each ambience was $25\,$

Superscript 'b' = number of broilers in each ambience was 200

*significant (p \leq 0.05) difference in comparison with respective control mean value

Mean values of each analyte at low (13-16 $^{\circ}$ C) and high temperatures (42-45 $^{\circ}$ C) were compared to respective values at control temperature 24-27 $^{\circ}$ C.

C-PTH= Carboxy terminal parathyroid hormone

Discussion

Pattern of changes of interrelated analytes showed the physiological state of the body being governed by endocrine system. During hot ambience all the parameters studied showed significant ($p \le 0.05$) changes while during cold ambience only few parameters showed significant ($p \le 0.05$) differences.

High ambient temperature

A significant increase in serum corticosterone at high ambient temperature (12) indicated that birds were stressed as was observed in mammals for cortisol (13). It was accompanied by increased concentrations of energy nutrients in sera like glucose, cholesterol, triglycerides and free fatty acids which substantiated the significance of corticosterone in meeting the energy crisis during stress. Increased concentrations of glucose, ASAT and ALAT indicated stimulation of gluconeogenetic process (6). Decreased levels of total serum proteins during hot ambience (14) along with increased creatinine, urea and uric acid levels also confirmed the role of corticosterone as proteolytic hormone. Increased serum lipase could result due to elevated levels of corticosterone as in mammals (15). Serum amylase activity should be interpreted in terms of higher urea and uric acid concentrations.

Reduction in serum T_4 and T_3 was probably to lower metabolic rate for thermoregulation (16) and to prevent hyperthermia. Low food intake during hot ambience was also correlated with circulating levels of thyroid hormones (17). Decreased levels of LDH and ICDH, the enzymes of tricarboxylic acid cycle (TCA) cycle were followed by an increase in serum concentrations of glucose, cholesterol, triglycerides and free fatty acids. Hepatic glycogenolysis and hyperglycaemia were reported in birds exposed to 48°C temperature(18).

Serum calcitonin level in the present study was higher than that in female birds (19) indicating the influence of testosterone on calcitonin (20). It was also higher than that in man, dog, pig, camel and cow (19,21 and 22). Calcium and calcitonin relation (23) in birds is well established but no literature could be traced on calcitonin level during higher environmental temperature. At 42-46°C ambient temperatures, lowering of calcium, phosphorus and magnesium could be attributed to increased level of calcitonin and lowered C-PTH. Hypocalcemic, hypophosphoric and hypomagnaesemic effects of calcitonin (24) could be due to stimulation of the calcium regulating axis by higher temperature (7). Low phosphorus during hot ambience could be related to low thyroid activity (25) and calcium metabolism. Low levels of calcium during hot ambience in the presence of higher calcitonin levels substantiated the significance of calcitonin as an emergency hormone to protect against hypercalcaemia (20).

The enzyme 5' nucleotidase and AKP increased during hot ambience. The determination of 5' nucleotidase is important in cases where serum alkaline phosphatase in increased. Higher glucocorticoid secretion is related with increased alkaline phosphatase activity (26). Increase in γ -GT could be attributed towards liver stimulation at higher temperatures. Serum γ -GT is possibly derived from high molecular weight fragments of liver that also contains AKP and 5' nucleotidase. Therefore AKP determinations should also be followed by γ -GT and 5' nucleotidase estimations.

Higher ambience induces dual stress, one of higher temperature and second of dehydration. Higher temperature stimulates adrenocorticotrophic hormone (ACTH) release which then regulates the secretion of aldosterone (27). Increased aldosterone level then helps in maintaining water balance. The results of aldosterone and sodium in present study indicated towards efficient handling of salt and water by the birds during conditions of stress. Higher aldosterone helped the birds to retain sodium in the body with increased sodium absorption from lower intestines (28) along with water reabsorption and decreasing potassium levels. Increased sodium and chloride during hot condition was not only due to haemoconcentration but also be due to increased aldosterone. Increased concentration of prolactin during hot ambience supported the earlier reportings that it is involved in important physiological functions (29) like avian salt and water balance (30).

Increased glucagon, corticosterone and growth hormone probably were responsible for increase in blood glucose. Increase in free fatty acids was probably due to lipolytic effect of growth hormone and glucagon. Glucagon served as stress hormone (31). During hot ambience secretion of enzyme pepsinogen decreased because of decrease in gastrin secretion.

Low ambient temperature:

Higher serum T_4 and T_3 during cold ambience could be due to modulation of thyroid activity by central hypothalamic-hypophyseal axis (32). Cold exposure in birds produces non-thermogenic shivering causing lowered glucose (31). Serum LDH and ICDH increased probably to increase the heat production. Significantly lower levels of aldosterone during moderate and low temperatures than hot could be due to low requirement of water for heat dissipation which increases blood volume resulting in a decline in aldosterone levels. During low ambient temperatures insulin concentration was higher with comparatively lower glucose, cholesterol, triglycerides and free fatty acids as it stimulates lipogenesis and inhibits the release of glycerol and free fatty acids in adipose tissue and stimulates conversion of glucose to fat. This was useful during cold ambience in increasing the peripheral utilisation of glucose for thermogenesis.

It was concluded that modulation of physiological mechanisms was for survival of birds during hot and cold ambience. Metabolic changes showed the endocrine regulation. An increase in corticosterone at higher temperatures denoted stress. Metabolic responses were reflected by the levels of proteins, cholesterol, glucose and fatty acids. Modulation in thyroid activity was a part of metabolic strategy for thermoregulation in varying ambience. Relationship of calcitonin with calcium, phosphorus and magnesium showed the variations in mineral metabolism. The study showed that calcium kinetics are not only important in hens but bears equal functional role in broiler chickens. It was clear that prolactin and aldosterone were important for salt balance. Serum enzymes levels indicated that their values require careful interpretation as changes may not necessarily be related with a pathology. Every laboratory should set the normal values of parameters of various types of birds found in the areas during different seasons.

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133

POVEZAVA MED TEMPERATURO OKOLJA TER VARIIRANJEM RAVNI HORMONOV IN POVEZANIH ANALITOV V SERUMU PIŠČANCEV IZ PUŠČAVSKIH PODROČIJ

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Povzetek: Za natančnejše razumevanje fizioloških mehanizmov smo raziskali razpon, v katerem se lahko spremenijo hormoni in povezani analiti v serumu brojlerskih piščancev ob spremembah temperature okolja. Zbrali smo kri piščancev, ko so bile temperature med 13 in16 °C (nizke), 24-27 °C (srednje) in 42-45 °C (visoke). Vrednosti analitov, ki smo jih izmerili v serumu, pridobljenem pri srednji temperaturi, smo jemali za kontrolne vrednosti, s katerimi smo nato primerjali rezultate iz obdobja nizkih in visokih temperatur.

Višje temperature okolja so povzročile večje variacije v analiziranih parametrih kot nižje. Pri 42-45 °C so se serumski kortikosteron, rastni hormon, glukagon, testosteron, sečna kislina, kreatinin, ureja, glukoza, holesterol, trigliceridi, proste maščobne kisline, ASAT, ALAT, lipaza in amilaza statistično značilno povečali ($p \le 0.05$). Tiroksin, trijodtironin, inzulin, gastrin, skupne beljakovine, albumini, globulini, LDH, ICDH in pepsinogen pa so se statistično značilno zmanjšali ($p \le 0.05$) glede na kontrolne srednje vrednosti. Pri nizkih temperaturah okolja (13-16 °C) so bile vrednosti serumskih T4, T3, LDH in ICDH občutno višje ($p \le 0.05$), vrednosti glukoze, holesterola, trigliceridov in prostih maščobnih kislin bistveno nižje ($p \le 0.05$), vrednosti kortikosterona, rastnega hormona, inzulina, glukagona, gastrina, testosterona, skupni beljakovin, albuminov, globulinov, sečne kisline, kreatinina, uree, ASAT, ALAT, pepsinogena, lipaze in amilaze pa se od srednjih vrednosti niso očitno razlikovale (p > 0.05). Nadalje so bili pri visokih temperaturah okolja povišani serumski prolaktin, aldosteron, natrij, klorid, kalcitonin, alkalna fosfataza, 5'nukleotidaza in gama glutamil transferaza, medtem ko so bile vrednosti kalija, C-PTH, kalcija, fosforja in magnezija očitno nižje. Pri nizkih temperaturah okolice so zelo padle serumske vrednosti aldosterona, natrija, klorida, alkalne fosfataze in 5'nukleotidaze, serumski kalij pa se je statistično značilno povečal ($p \le 0.05$) v primerjavi z vrednostmi pri srednjih temperaturah.

Ugotavljamo, da temperatura okolja vpliva na presnovo glukoze, maščob, beljakovin, kalcija in natrija, in sicer tako, da omogoča pticam preživeti zelo raznolike okoljske razmere. Vzorec sprememb medsebojno odvisnih analitov kaže na endokrino uravnavanje fiziološkega stanja organizma.

Ključne besede: temperatura okolja; analiti; serum; brojlerji; hormoni

YERSINIOSIS IN CAPTIVE CAPYBARAS (HYDROCHAERIS HYDROCHAERIS)

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Summary: Four young female capybaras (*Hydrochaeris hydrochaeris*), three to four months old, died in the late autumn of year 2001 in the Ljubljana Zoo. Three of them were submitted for dissection. Granulomatous lesions in small intestine and mesenteric lymph nodes with severe haemorrhagic enteritis and ascites were found in all capybaras. Microscopically pyogranulomatous enteritis, lymphadenitis, hepatitis, splenitis, and pneumonia were diagnosed in two capybaras and pyogranulomatous enteritis, lymphadenitis, hepatitis, splenitis and diffuse fibrinopurulent pneumonia in one. *Yersinia pseudo-tuberculosis* was isolated from small intestine, liver, lung and spleen from all dissected capybaras.

Key words : Capybara (Hydrochaeris hydrochaeris); pathology; pyogranulomatous lesions, Yersinia pseudotuberculosis

Introduction

Yersiniosis is an infectious bacterial disease caused by *Yersinia pseudotuberculosis*, a small, gram negative, aerobic, pleomorfic coccobacillus, which causes disease in rodents, lagomorphs, nonhuman primates (1, 2), birds, carnivores, ungulates, reptiles and many other animal species (3, 4, 5, 6, 7, 8) including humans (9, 10, 11). Among free-living animals yersiniosis appears to be an important disease of hares (12) and doves (13), but the most affected seem to be animals from the Zoos and wildlife parks (3, 4, 5, 8, 14).

Infection with *Y. pseudotuberculosis* is usually acquired orally, while feeding with food, contaminated with feces of carriers, mostly rodents and birds (5, 13, 15).

We described pathomorphological lesions in naturally occurring yersiniosis in three capybaras (*Hydrochaeris hydrochaeris*) from Ljubljana Zoo. Capybaras belong to the order Rodentia, which is very susceptible for yersiniosis although to date only a few cases were reported but not described in this animal species (4, 16).

Materials and methods

Capybaras were dissected at the Institute of Pathology, Forensic and Administrative Veterinary Medicine of Veterinary Faculty in Ljubljana. Representative specimens of spleen, liver, mesenteric lymph nodes, small intestine, lungs, kidneys and myocardium were fixed in 10% neutral buffered formalin for 24 hours, routinely embedded in paraffin, sectioned at 4 μ m and stained with hematoxylin and eosin (HE).

Samples of spleen, liver, mesenteric lymph nodes, small intestine, lungs and kidneys were inoculated on blood agar (nutrient agar Oxoid, Unipath Ltd., Basingstoke, UK, supplemented with 5% of ovine blood) and Drigalski agar. Inoculated plates were incubated at 37° C for 48 hours, even though the colonies were visible after 24 hours of incubation. Subcultures for Y. pseudotuberculosis identification were also prepared on blood agar. Bacteria that had grown on culture media were Gram stained (Gram Stain Kits and Reagents, Becton Dickinson and Company, USA), and tested for catalase and oxidase activity (Difco-BBL). The strain was biochemically characterised by using commercial system API 20 E (API bioMerieux, Marcy l'Etoile, France). Enzyme reactions were read after 24 hours of incubation at



Figure 1: Intestine with mesenterial lymph nodes. Pyogranulomatous lymphadenitis caused by *Yersinia pseudotuberculosis*



Figure 2: Lung. Pyogranuloma with Yersinia pseudotuberculosis colonies in the centre. HE staining, $x\,200$



Figure 3: Liver. Necrotic focus with large Yersinia pseudotuberculosis colonies in the centre. HE staining, x 400

37° C according to the analytical profile index. Additional test for aesculin hydrolysis was performed classically. In vitro susceptibility of the bacterium to antimicrobial drugs by the agar disc-diffusion method (Kirby-Bauer) was performed.

Strain of *Y. pseudotuberculosis*, isolated from samples obtained from inter-laboratory control scheme (QA), was used as reference strain for media growth control.

Results

Anamnesis

Four young female capybaras from a family, composed of two parents and a litter of five offspring, which lived in an enclosure near the fish pond and were feed predominantly with vegetables, died in the Ljubljana Zoo in November and December 2001 during one month period. Three of them were submitted for dissection. Two capybaras were three months old and they died suddenly, one was four months old and was anorectic and depressed with fever and severe diarrhea two days prior to death. We don't have information about clinical signs and status of the fourth capybara, which derived from the same litter, but was not submitted for the necropsy.

Necropsy

All capybaras were in good condition. Gross lesions were similar in all animals: the peritoneal cavity contained one liter of transudate and the peritoneal surface was translucent and smooth. The small intestine and enlarged mesenteric lymph nodes, with diameter up to three cm, were scattered with numerous nodular, grey-whitish necrotic lesions, from few millimeters to one cm in diameter (Figure 1). The small intestine was severely inflamed and lumen was filled with dense, bloodstained content. The large intestine exhibited catarrhal inflammation and contained brown, dense feces. The spleen, liver, lungs and kidneys were hyperemic.

Histopathology

Microscopic examination revealed some differences in a form and a degree of pathological lesions among three dissected capybaras.

Widely extended areas of necrosis and hemorrhages, with numerous bacterial colonies in the very centre, densely infiltrated with necrotic mixed inflammatory cells, heterophils and macrophages were found in the small intestine's mucosa, edematous and hemorrhagic submucosa, lungs and spleen in the first capybara (Figure 2). Necrotic foci, with one or several large bacterial colonies in the centre, surrounded by necrotic mixed inflammatory cells and some heterophils were scattered also throughout the muscular layer of the small intestine, mesenteric lymph nodes and liver (Figure 3). Several small bacterial colonies were noticed also in the renal glomeruli.

The pathological lesions in the small intestine of the second capybara were similar to those described in the first one, with some differences in location of the lesions: several segments had lesions only in mucosa; in others all intestinal layers were affected. Widely extended necrotic and hemorrhagic areas, with numerous bacterial colonies, densely infiltrated with remnants of mixed inflammatory cells and surrounded by heterophils and macrophages were noticed in mesenteric lymph nodes. Only few necrotic foci, with some necrotic mixed inflammatory cells, heterophils and rare macrophages were found in lungs, liver and the spleen, and only those in lungs contained bacterial colonies.

The small intestine mucosa and submucosa of the third capybara were also scattered with multifocal necrotic areas, in which remnants of mixed inflammatory cells and heterophils were noticed, surrounded by thin fibrous capsule, infiltrated with macrophages and lymphocytes. Rare bacterial colonies were located at the periphery of described lesions. Similar lesions were more frequent and confluent in mesenteric lymph nodes and less frequent in spleen and liver, where there were no bacterial colonies. In lungs, extended areas of fibrinopurulent pneumonia with necrotic foci, containing heterophils and small bacterial colonies, were found.

Described lesions were evaluated as acute pyogranulomatous enteritis, lymphadenitis, hepatitis, splenitis and pneumonia in the first two capybaras and subacute pyogranulomatous enteritis, lymphadenitis, hepatitis and splenitis with diffuse fibrinopurulent pneumonia in the last one.

No patohistological lesions were found in the myocardium of dissected capybaras.

Bacteriological culture

Bacteriological culture of mesenteric lymph nodes yielded abundant growth of small colonies, non-hemolytic, grayish, shiny and similar to those of the *Enterobacteriaceae*. Colonies on Drigalski agar were blue-green color due to their inability to ferment lactose. Colonies on blood agar and Drigalski agar as well were smaller than those of the other members of *Enterobacteriaceae* after 24 hours of incubation. The same type of less numerous colonies were also isolated from almost all the other examined tissue (liver, spleen, lung, intestine), but not from kidney. Gram stain of culture demonstrated Gram-negative rod-shaped bacteria. On the basis of the cellular morphology and biochemical profiling the isolate was identified as *Y. pseudotuberculosis*. Isolated strain was susceptible to amoxycillin/clavulanic acid and enrofloxacine, but resistant to neomycin and trimethoprim/sulphametoxazol.

From intestine, non-hemolytic *Escherichia coli* and alpha-hemolytic streptococci were also isolated.

Discussion

Zoo animals are very susceptible for yersiniosis, especially birds, primates, ungulates and rodents (1, 3, 4, 5, 13), but in the literature we found only descriptions about one case of yersiniosis in capybara from Bristol Zoo (4) and seven from Berne Zoo (16). In the Ljubljana Zoo there was one outbreak of yersiniosis, in which only capybaras were affected.

All capybaras died in the late autumn, which was cold and rainy in 2001. Several authors described yersiniosis as a seasonal disease with outbreaks occuring mostly in the late autumn and winter, from November to April (1, 3, 5, 8, 14). Unfavorable weather conditions, i.e., cold, rainy weather and floods, increase animals susceptibility (1, 8, 17).

All capybaras were young females, at the age of three to four months. There were no information about capybaras with yersiniosis from Bristol Zoo (4) and Berne Zoo (16), but we think that young animals are more susceptible for infection due to immature immune system. In the literature there are no data concerning sex predisposition for yersiniosis and we also don't know the gender of survived offspring from Ljubljana Zoo.

Several authors think that food, contaminated with feces of rodents and birds, the well-known carriers of bacteria *Y. pseudotuberculosis*, is the most possible source of the infection (5, 13, 15). In the Ljubljana Zoo, capybaras' habitat is located near the artificial pond, where the concentration of rats is the highest. Taking in consideration the results of others we can summarize that the infection in the Ljubljana Zoo was probably transmitted by rodents. In Ljubljana Zoo the first two capybaras died without clinical signs and only in the third one diarrhea, anorexia, emaciation and fever were noticed two days prior to death. Data about clinical signs are not known for the fourth capybara, which was not submitted for the necropsy. All three dissected capybaras were in good condition. There are no pathognomonic clinical manifestations of yersiniosis (3). Infected animals may die suddenly, without any clinical signs (8, 14), although fever, diarrhea, anorexia, depression and emaciation were often reported in animals, infected with *Y. pseudotuberculo-sis* (1, 3, 5, 8).

At necropsy nodular lesions were found only in the small intestine and mesenteric lymph nodes in all capybaras. Similar nodular lesions were often found also in liver, spleen, lungs and kidney in different animal species (3, 4, 5) and in the myocardium of a guinea pig (1). Distribution, expansion and number of lesions depend primarily on the animal species (13) and probably also on the length of survival time of the infected animal. Unfortunately there were no data about the pathological lesions in yersiniosis in capybaras from Bristol Zoo (4) and Berne Zoo (16).

Histopathological lesions, described in the first two capybaras, show an acute form of yersiniosis, which was described in primates (2) and ungulates (8, 13). In the third capybara the pathological lesions were surrounded by thin fibrous capsule and were evaluated as subacute. The subacute form of yersiniosis was reported in birds and several mammals (8, 13). In chronic form of the disease, described in some rodents, manly in rats, granulomas in liver, lungs, kidneys and lymph nodes, but not in the intestine were diagnosed (8, 13). We believe that yersiniosis in capybaras probably started in small intestine and spread through the mesenteric lymph nodes to lungs, liver and the spleen. Capybaras probably died of septicemia.

Y. pseudotuberculosis was isolated from intestine, liver, lungs and spleen of all capybaras. Bacterial colonies on the blood agar were small, round and grayish. Colonies, described in literature, were also round, grayish, with opaque center (8).

Treatment of yersiniosis is due to late onset of clinical signs or their absence often unsuccessful (5). It was ascertained that some isolates were susceptible to several antibiotics, including penicillin, streptomycin, gentamicin, sulphadimethoxin, neomycin and trimethoprim/sulphadiazine (14). *Y. pseudotuberculosis* is also susceptible to tetracyclines (13). Prophylaxis with sulphonamides (1) and tetracyclines (8, 14) is recommended. Several authors believe that adequate feed and drinking water hygiene with preventive measures towards birds and rodents are of great importance in the prevention of yersiniosis (1, 5, 8, 14). In our study was established that isolated strain of *Y. pseudotuberculosis* was susceptible to amoxycillin/clavulanic acid and enrofloxacine, but resistant to neomycin and trimethoprim/sulphametoxazol. Enrofloxacine was immediately applied to all survived capybaras and a deratization was carried out in the whole Zoo. Till date no outbreaks of yersiniosis in capybaras or in any other animal species in the Ljubljana Zoo were diagnosed.

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JERSINIOZA PRI KAPIBARAH (HYDROCHAERIS HYDROCHAERIS) IZ ŽIVALSKEGA VRTA

M. Gombač, T. Švara, I. Zdovc, P. Juntes, M. Pogačnik

Povzetek: V Ljubljanskem živalskem vrtu so jeseni 2001 poginili štirje mladiči kapibar (*Hydrochaeris hydrochaeris*) ženskega spola, stari od tri do štiri mesece. Tri od njih smo prejeli v raztelesbo, pri tem pa smo ugotovili granulomatozne spremembe v tankem črevesu in mezenterialnih bezgavkah kakor tudi hemoragično vnetje črevesa in ascites pri vseh raztelešenih kapibarah. S patohistološko preiskavo smo pri dveh kapibarah diagnosticirali piogranulomatozno vnetje črevesa, bezgavk, jeter, vranice in pljuč ter piogranulomatozno vnetje črevesa, bezgavk, jeter, vranice in difuzno fibrinopurulentno vnetje pljuč pri eni od kapibar. Z bakteriološko preiskavo je bila iz tankega črevesa, jeter, pljuč in vranice vseh kapibar izolirana bakterija Yersinia pseudotuberculosis.

Ključne besede: Hydrochaeris hydrochaeris; patologija; priogranulomatozno vnetje; Yersinia pseudotuberculosis

ON THE OCCASION OF 200TH ANNIVERSARY OF THE BIRTH OF DR JANEZ BLEIWEIS

EDITORIAL

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This year marks the 200th anniversary of the birth of a great, though under-known Slovene, Dr Janez Bleiweis. He was born in 1808 in Kranj and was a doctor, veterinary surgeon, journalist and politician by profession and interest, who was dedicated to the progress of Slovenia and development of the Slovene language. Slovenian Veterinary Research has published a special issue dedicated to this great Slovene, who has majorly contributed to the development of medical and veterinary education in Slovenia and to the development of Slovene scientific terminology. However, since work of Janez Bleiweis was important not only locally, we decided to publish abstracts of articles from this special issue also in a regular issue of Slovenian Veterinary Research, which is distributed internationally, so we can introduce Janez Bleiweis to the international audience.

Janez Bleiweis made a great contribution to the development of the standard Slovene language and to national awakening, to developing consciousness of the fact that the Slovenes are a nation and as such deserve our own country. Although Janez Bleiweis graduated in human medicine in Vienna and was at one point a director of the Ljubljana maternity hospital and professor of forensic medicine at Ljubljana Medical-Surgical institute, he devoted more of his time to veterinary medicine. His mission was the progress of the Slovene people. For him the most important part of a nation was farmers and craftsmen whom he saw as the basis of the economy and thus a founding element for the existence of a nation and state. Already then he knew that farmers and craftsmen can only be successful if they are educated and knowledgeable. Therefore as the secretary of the Agricultural Society of Carniola he strove for the establishment of a three-year agricultural school. He was successful in his endeavors and in 1848 a veterinary and farrier school was established in Ljubljana, with its first students enrolled in 1850. Thus Janez Bleiweis was the originator of veterinary education in Slovenia and a predecessor of veterinary experts at the Ljubljana Veterinary Faculty, which was established over a hundred years after his school. In 1843 Janez Bleiweis started publishing a newspaper entitled Kmetijske in rokodelske novice (Farmers' and Craftsmen's News), whose editor and main journalist he remained until his death in 1881. For a number of years it was the only regular newspaper published on the territory of the then Carniola and it brought important advice to farmers and craftsmen for »wiser management«. Janez Bleiweis was the first publisher

and editor of a specialist newspaper in the Slovene language. Bleiweis definitely deserves a lot of credit for the development of the Slovene standard language. As a politician he supported the introduction of Slovene into schools and offices and was instrumental in the adoption of Gaj's alphabet, which is still in use today. In his work he was pragmatic and in favour of slow changes. He was not one to support revolutionary change, which is probably the main reason why he was so underrated in our recent history. He was also religious and wanted the Church to play an important role in the society. Besides, Bleiweis strongly opposed the Illyrian movement and thought the ideas of a uniform language of the South Slavs, which some of his contemporaries supported, to be inappropriate as such a language could not exist since it would be an artificial formation. For his commitment to the Slovene language and his editing and publishing the Farmers' and Craftsmen's News Janez Bleiweis can be considered the first publisher of specialist literature in veterinary medicine. In a way his News can be seen as a predecessor of today's Slovenian Veterinary Research journal. Since its beginning in 1961 the Research has seen numerous changes and as a scientific publication does not have the same readership and mission as the Farmers' and Craftsmen's News did. They have something in common though - despite globalisation and supremacy of the English language in science all over the world, the editorial board of the Slovenian Veterinary Research still strives for the preservation and development of the Slovene scientific language. Even though some years ago the Slovene version of the Research was discontinued, all articles, including those by foreign authors, are still accompanied by summaries in Slovene, which is our way of nurturing and preserving our scientific language. This year, perhaps symbolically at the 200th anniversary of the birth of Dr Janez Bleiweis, the Slovenian Veterinary Research received a special acknowledgement by being accepted in a reputable group of journals which have their citation index determined, on the basis of which a journal's impact factor is awarded. Thus the Slovenian Veterinary Research earned a great reputation of being the first scientific journal on biomedicine in Slovenia to manage to break through into this select group of journals from all over the globe, which is, unsurprisingly, dominated by journals from English-speaking countries. By being included in the Science Citation Index, the Slovenian Veterinary Research will gain global recognisability, which in the age of globalisation is very important also for small journals and nations. At the 200th anniversary of the birth of Dr Janez Bleiweis the editorial board finds it fitting for our journal to render homage to this great Slovene, by publishing a special edition in which different authors will try to shed light on his

figure and work, which was very important for all of us to be able to live in our own country and be educated in our own language. In this way we wish to pay our respects to the memory of Dr Janez Bleiweis and bring him and his significance for the Slovene nation a little closer to all those who may not be familiar with his work.

JANEZ BLEIWEIS - ON THE 200th ANNIVERSARY OF HIS BIRTH

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Much has been written about the political, professional, cultural and editorial work of Dr Janez Bleiweis in the last decades and authors of different disciplines have convincingly refuted a dark and negative image that literary and political historians had, since the beginning of the 20th century, created and spread concerning Bleiweis, his political, cultural and historical role and his spiritual horizon. Researchers who re-examined and reconsidered Bleiweis' activities in national politics today agree that until his death Bleiweis remained a central figure and an undisputed leader of the Slovene national movement. He was - in Vasilij Melik's words - the first Slovene politician, for he was a politician when there was no Slovene politics and enjoyed a good reputation as a politician after Slovene politics had acquired a more definite direction and characteristics. He was among the first to accept the name 'Slovenia' for the territory inhabited by the Slovene population and with his News decisively influenced the adoption of a uniform Slovene alphabet and, cautiously yet persistently, strove to bring about the equality of the Slovene language and Slovene national demands. His critics liked to mock the title of "the father of the Slovene nation", yet for most of his contemporaries who used it, it was an expression of genuine acknowledgement and respect. This fact was well known even to Bleiweis' opponents, who - despite criticism - acknowledged his merits in the development of Slovene national consciousness, his efforts to raise the educational level of the population and modernise the countryside as well as his endeavours for unified national political action. Those who know his work well have long maintained that he was a good and knowledgeable expert, who with his professional articles, organisational and educational activities and a number of practical proposals and initiatives was an untiring advocate of the modernisation of the Slovene society and economy and informed his compatriots of the latest achievements in agriculture, especially stockbreeding, veterinary medicine and medicine, i.e. in the areas he was engaged in.

According to Professor Srdjan Bavdek, two hundred years after Bleiweis' birth these opinions and findings are, like in the present volume of Research, more or less known and accepted only in narrow scholarly circles, while in the memory and consciousness of a broader public a negative image of Bleiweis remains, an image of a frightened, conservative, even clerical politician, who in his loyalty to the emperor and monarchy opposed any more decisive national political initiatives and far-reaching cultural and economic ideas. This image was mostly instilled by political and literary historians, who besides his conservatism resented Bleiweis' allegedly unfavourable attitude towards Prešeren, his – also alleged – ties with clericalism and ultramontanism and his disputes with the Young Slovenes, and it was politically generalised and aggravated by Edvard Kardelj in the 1930s in his work Razvoj slovenskega narodnega vprašanja (The Development of the Slovene National Question). Such decidedly negative and historically distorted image entered history books and textbooks after the Second World War.

The papers published in this commemorative volume of Research refute the negative assessments of Bleiweis' personality and work, which persisted for so many years. Unkind and adverse views of Bleiweis have, according to Srdjan Bavdek's extensive examination of judgments of "the father of the Slovene nation" from different periods, their roots in severe political polarisation between the Young Slovenes and Old Slovenes in the 1860s and 1870s, and it was critics' political, literary and historical views rather than an objective analysis and reflection that influenced the creation of a one-sided and dark image of Bleiweis in the previous century. Certainly Bleiweis did not only attract negative criticism during his life and later on in the twentieth century; there were positive opinions as well, although the former, under the influence of political circumstances and development, gradually overshadowed the latter. The articles collected in this volume illuminate Bleiweis' political, editorial, educational, professional and cultural work from different angles, convincingly argue against negative views of his historical role and importance and objectively place his person, points of view and work into the historical concept of the time in which he lived and worked. In this light they present him as a doctor, veterinary surgeon, agricultural expert, writer, organiser of vocational education, and secretary of the agricultural society, and examine his efforts in stockbreeding, forensic medicine, eradication of livestock infectious diseases, organisation of veterinary service and development of scientific terminology. Srdjan Bavdek also discusses Bleiweis' political involvement and his views of the language, Prešeren and literature and points out that the theses of Bleiweis' clericalism and his unfavourable attitude to Prešeren have long been disproved in modern historiography and literary history.

Therefore this commemorative volume is an account of Dr Bleiweis' figure and work based on findings of the most recent research, which reveals in no ambiguous terms that Bleiweis and his adherents had a rather clear and recognisable programme. It was based on one hand in the enlightenment, reform and physiocratic movement of the Pre-March Period and on the other hand in conservative yet realistic and gradual modernisation-oriented view of Slovene cultural, social and economic conditions. Bleiweis' focus was farmers and small craftsmen, whom he saw as the most important agents of Slovene national consciousness. He was against more radical social changes, which could increase social tensions, cause pressure on towns and pave the way for Germanisation. He believed that a hurried transformation of what was then still predominantly rural society could seriously threaten the future of the Slovene nation. In accordance with these views of Slovene opportunities and needs he tried to direct Slovene politics and avoid divisions and extremes in political life.

The authors of this volume of the Slovenian Veterinary Research summarise and comment on what was written and said about Bleiweis during his life, in the periods that followed and in last decades. In this light they convincingly portray him as one of the central characters of the Slovene history of the 19th century, who must not sink into oblivion.

SOME REFLECTIONS ON THE FIGURE AND WORK OF THE FATHER OF THE SLOVENE NATION

Srdan V. Bavdek

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While Dr Janez Bleiweis (1808-1881), a doctor and veterinary surgeon, enjoyed the reputation of "the father of the Slovene nation" among his contemporaries, he was also a target of quite some criticism, which in the 20th century intensified and consequently diminished, belittled and pushed into oblivion the former leader's reputation. We managed to refute a number of these criticisms. We have found that Bleiweis was determined in his efforts for the Slovene language and the nation's rights as well as in defending the autonomy of knowledge (reason) and in disagreeing with dogmatisms (such as clericalism). He was correct and measured in his attitude to religion and politically cautious (pragmatic) towards both secular and church authority. Such structuring of personal characteristics suited his programme orientation and desires.

Bleiweis was the first to comprehensively connect national revival with national movement. He gave the national revival program of the enlightenment circle of the late 18th century concrete substance in cultural, economic and political areas. The physiocraticutilitarian national programme of the enlightenment was given fresh impetus and was largely realized between 1843 and 1881, thanks to Bleiweis. He can be counted among the most active, committed and consistent agents of social development in the history of the Slovene language.

In our recent historiography Bleiweis has been called our first and only politician (already) at the time when the absolutist regime would not allow any. The second part of this claim gives the circumstances, while the first one is substantive. In it one can see national emphasis and pioneer significance. Bleiweis is therefore the leader of our political and social life on the national basis. This is consistent with the title of "the father of the nation".

In or discussion we devoted special attention to the relationship between Bleiweis and the poet France Prešeren (1800-1849), who is seen as the central figure of the Slovene culture. In the context of our discussion it is only reasonable to join the historic messages of Prešeren and Bleiweis, into a solid notion of the national being of the Slovenes and their common house, which was built on the foundations of the nation's self-assertion and a strong desire for coexistence in a common state.

FORENSIC MEDICINE - AN UNKNOWN SIDE OF DR JANEZ BLEIWEIS

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Most Slovenes see Dr Janez Bleiweis as a conservative politician and representative of the Old Slovene movement, which resisted the development of the Slovene language and remained loyal to the Emperor's / King's court law. His enlightenment work as editor of Kmetijske in rokodelske novice (Farmers' and Craftsmen's News) is also well known among Slovenes. What is not widely known is that he was a reputable doctor and professor of veterinary medicine and forensic medicine at the Ljubljana Medico-surgical Institute. There are no written sources about his forensic work and we can only surmise that he may have participated in court proceedings as an expert. He preserved for us the language of forensic medicine of that era, as from mid-September to early December of 1852 the News published the Slovene text of the penal code used at the time. The translation of the penal code was not his work. Slovene forensic medicine started developing about fifty years later, when forensic expert opinions of F. Zupanc, P. De Franceschi and A. Homan were published. For his great contribution to the development of veterinary medicine and education, Bleiweis definitely did not deserve the disregard and neglect that he seems to receive. His work at the Medico-surgical Institute and later his establishment of a veterinary and farrier school should ensure that this proud, if unrebellious, Slovene is remembered.

MEDICAL AND VETERINARY EDUCATION IN THE AUSTRO-HUNGARIAN MONARCHY UP TO THE 19TH CENTURY Andrej Pengov

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We usually judge the figure and work of Dr Janez Bleiweis by his endeavours for the rise of our literature, his role in the politics of the time and his merits in veterinary medicine and agriculture in Carniola. It should not be overlooked, however, that Dr Bleiweis was primarily a doctor by profession, which was at that time and up until the end of the 19th century the basic precondition for entering a two-year course in veterinary medicine. Those who successfully completed the course were awarded Bachelor's degrees in veterinary medicine and, after 1841, Master's degrees in veterinary medicine. Surgeons as well could enrol in the course and were upon its completion awarded the same degree, which in Bleiweis' time allowed Masters of veterinary medicine to practice on all animal species all over the monarchy. The paper presents in detail the development of the university and particularly the medical faculty in Vienna, which, from its foundation to the end of the First World War, educated the majority of doctors and, from the end of the 18th century onwards, almost all veterinary surgeons in the area of today's Slovenia.

JANEZ BLEIWEIS AND CATTLE PLAGUE

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Cattle plague is a severe viral disease of ruminants with high incidence and morbidity rates, especially in thoroughbred cattle. The disease is known from history and caused most damage in the 18th and 19th centuries. In one of the last epizooties on our territory, which affected Carniola in the early 1860s, cattle plague was fought and studied by Dr Janez Bleiweis, a veterinary surgeon of the province of Carniola. He carried out an experiment on live animals, which served to prove that cattle and sheep plague were the same disease. Considering the course of the disease, especially as regards its spreading, an attempt to transmit the contagious material from sheep to cattle was the only appropriate step. Thus Bleiweis achieved several goals, i.e. he confirmed the assumption of experts that sheep indeed had cattle plague and convinced the authorities as well as stockbreeders, traders, butchers, etc. to apply the strict disease prevention measures used at cattle plague outbreaks to sheep as well. With his work Dr Janez Bleiweis gained appreciation in scientific circles and definitely contributed to gradual eradication of cattle plague in our country and other European countries.

well as clumsiness have disappeared. It can be safely concluded that Dr. Bleiweis left behind a significant contribution to introduction and consolidation of professional terminology in Slovenian language.

BLEIWEIS - A PROPONENT OF STOCKBREEDING Jože Osterc

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Bleiweis was a great patriot and physiocrat. Both contributed to his moving from Vienna to Ljubljana, where he became the secretary of the Agricultural Society of Carniola in 1842 and editor of Kmetijske in rokodelske novice (Farmers' and Craftsmen's News) in 1843. Both functions enabled and forced him to concentrate on stockbreeding problems and their solutions. He was aware of the importance of good broodstock. As the secretary of the Agricultural Society he supplied progressive farmers with financial support for buying good sires. He knew how important knowledge was for the development of stockbreeding so he promoted the foundation of general agricultural schools for farmers and landowners. He also taught at these schools. He published a number of works on agriculture. In 1858 he wrote and published Nauk od reje domače živine (The Science of Animal Husbandry), which constituted part three of Živinozdravstvo (Veterinary Medicine). In 1871 the Agricultural Society of Carniola commissioned him to write Nauk o umni živinoreji (The Science of Sound Stockbreeding). Most of the book focused on cattle and horses, while other stock received much less attention. Breeds of horses, cattle and sheep that were most important for our areas were presented in pictures as well. He also mentioned the significance of manure of particular livestock species.

DR JANEZ BLEIWEIS, HIS CONTRIBUTION TO THE EVOLU-TION OF THE SLOVENE PROFESSIONAL TERMINOLOGY Jože Jurca

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The work of Dr. Janez Bleiweis in the domain of Slovenian professional terminology and expression is in fact a continuation of endeavours of Zois circle and a reactivation of Slovenian professional medical and even more veterinary language. His basic motto regarding literary as well as professional language was, that the written word has to be understandable, therefore local, domestic. When need arises, it can be judiciously complemented from foreign vocabularies, especially from Slav languages. In his early writings a similarity to older literary models, especially those from the age of enlightenment, can be observed. These writings contain a good number of distorted germanisms and clumsy Slovenian expressions, but on the other hand very many new Slovenian professional terms, which have hold ground upto today. In his later writings the language becomes clearer and more fluid, the professional terms have consolidated, and foreign words as

THE WORK OF DR. JANEZ BLEIWEIS AND HIS PERSONALITY Srdan V. Bavdek

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The first part of the treatise explains Bleiweis's endeavours in the field of Slovenian literary language and his endeavours for progress in agriculture and handicrafts. It also lists the conferred honours, of which the most notable one is the Trsteniški Knight. The treatise contioues with a brief description of his lineage and studies in Vienna, his work at the medical surgical institute in Ljubljana as professor of veterinary medicine (1841-1850), his medical work in Carniola and his endeavours in encouraging the establishment of a university in Ljubljana. The organisation and management of the farriers' and veterinary school in Ljubljana (1850-1881) is further explained, likeweise the organisation of veterinary services of the time, and Bleiweis's activities connected with his post as provincial veterinarian for Carniola (1856-1874), as well as his attempts to promote knowledge and prudent farming. There is a brief account of his professional

works related to veterinary medicine and animal breeding, and of his activities as secretary of the agricultural society of Carniola (1842-1881). This is followed by a description of his contribution to storytelling and of his political activities, particularly those connected with the Unified Slovenian programme, which led him to become one of the most distinguished Slovenians of the 19th century and the father of the Slovenian nation. Bleiweis helds a permanent place in the Slovenian literary history for being the editor of the newspaper Kmetijske in rokodelske novice (Agricultural and Handicraft News; 1843-1881) -the sole Slovenian organ existing at the time of its establishment. He was among the founders of the Slovenska matica (1864), an extremely important foundation for the development of Slovenian culture, and its third president (1875-1881).

Dr. Janez Bleiweis, born on the 19th November, 1808 in Kranj, died on the 29th November, 1881 in Ljubljana, attended elementary school in Kranj between the years 1815 and 1819 and grammar school in Ljubljana 1819-1824, where he also completed a two-year study of philosophy at the lyceum. In Vienna he finished his studies of medicine in 1832 with his dissertation on De hirudine medicinali. The following year he finished a two-month practical course in obstetrics and was trained for vaccinating against smallpox. He continued the two-year study of veterinary medicine (1833-1835) and received his diploma in this subject. In 1834, he took up the position of a probationer at the veterinary institute in Vienna, in January 1836, however, he became a private totor, For the needs of the veterinary school in Vienna he wrote a text-book on internal horse diseases.

In 1841, he was given the chair of veterinary medicine at the medical surgical institution in Ljubljana and received the title of professor. He also had to undertake lectures on forensic medicine. He was performing both duties until 1850, when the medical surgical institution closed down. In 1851, he retired, retaining the title of professor from the time when he was teaching at the farriers' and veterinary school. In 1856, Bleiweis resumed work, taking up the post of provincial veterinarian, and in 1874 he retired with this title.

On April 13th, 1848 the Ministry of education proclaimed the farriers' and veterinary school in Ljubljana (officially named Hufbeschlag Lehranstalt und Tierspital) as a public school, they confirmed the proposed curriculum of the school and gave dr. Janez Bleiweis and dr. Simon Strupi permission to teach there. Bleiweis was appointed as head of the school. The school officially started to operate on January 7, 1850. Lessons were given entirely in the Slovenian language and free of charge. The school had a double programme, namely for farriers (6-/month training course) and for veterinary assistants (one-year training course). Bleiweis wished to elevate the veterinary programme to a two-year course for the training of district veterinarians, but the gov-rnment abolished the training of veterinary assistants in 1881, whereas the farriers' course continued. Thus, the training of veterinary experts in Slovenia lagged behind for quite a considerable period of time.

Bleiweis's research into sheep pest (Die Identitat der Rinder- und Schafpest zum ersten Male durch Impfversuche constatirt; Wien, 1864) is important, while his technical texts, written when working in Ljubljana, can be classified into three periods. In the first period, 1843-1850, he wrote a popular technical book "Bukve za kmeta" (Books for the farmer; 1843), and published articles in the newspaper Novice about pest diseases, and about hygienic or zoohygienic issues. A period of writing followed focusing on encyclopedic work "Živinozdravilstvo" (Veterinary medicine), to aid pupils attending the school of horseshoeing and veterinary medicine, whereby he shared the work with Strupi. In the third period, from time to time Bleiweis published articles covering topical issues in Novice and directed much of his attention to articles on animal breeding, reaching the peak with his work "Umna živinoreja" (Instructions for prudent animal breeding; Ljubljana, 1871).

Bleiweis was active also in the field of health service. In 1851, he became a member of the permanent health committee for Carniola, in which he worked until the public health service in Carniola was reorganised in 1870. In 1860, he was appointed as temporary provincial health councillor, and was thus for a while at the same time performing both the health care and veterinary services of the highest rank in the country. In 1861, he was acting as director of the maternity hospital in Ljubljana. He would be very convincing in the provincial assembly when health issues were in question. He gratly contributed to the decision made by the provincial assembly that the maternity hospital should be expanded and a provincial mental asylum built. He also wrote about health issues.

As secretary of the agricultural society Bleiweis and his collaborators disseminated the views established in the period of Enlightenment and physiocracy; they endeavoured to increase the fertility of soil and the produce. The doctrine about the urgent structural change in farming was realised in Bleiweis's time: the transition from corn farming to animal breeding was carried out. This change, along with the distribution of the village and common land pastures, had a positive effect on crop rotation, increase the produce and led to the introduction of growing potatoes, maize and fodder plants. Higher yields were gradually being achieved in farming, while the efficiency of live labour improved and the reputation of the agricultural society grew. To ensure the implementation of the programme set by the agricultural society, he prepared a number of works, mainly translations. His major contribution related to the development of animal breeding and schools of agriculture.

The contacts established among all the Slovenians through the journal Novice, reporting on topical public issues, were of primary importance to the national movement; the Novice became the hearth of Slovenia. Bleiweis succeeded in raising this organ from an insignificant agricultural organ to the rank or role of a teacher -leader of the Slovenian nation. A good number of collaborators gathered around him and with joint efforts the journal's repute spread across the Slav world. The Novice journal was some kind of a universal Slovenian magazine and a true repertoire of our literature in the years 1843-1858, namely, a journal of the Slovenian nation experiencing regeneration. Bleiweis composed Slovenian readers for lower classes of middle schools and issued theatrical plays, chronicles and calenders.

Bleiweis's activities, aiming towards a unified Slovenia, show that he sought solutions in a progressive and legitimate way. We are perhaps not fully aware of the importance of his influence on the fact that the Slovenian nation did not diverge from the main road leading to progress, and that the nation's rights and claims had not been enforced in a manner which would isolate this nation from other nations; that the Slovenian national issue did not beeome an exclusivity, a matter of a closed circle of people, something marginal or extreme; that the nation was able to unite at the right moment of decision. Anyhow, Bleiweis's endeavours had not at the time yielded practical solutions, but were brought about only in the new 20th century.

In Ljubljana Bleiweis had several political functions. In the period between 1961 and 1878 he was a permanent member of the provincial assembly, elected seven times successively, in the years 1871 and 1872 he was replacing the governor general in the state council, and in the period 1878-1881 the emperor appointed him as deputy governor general.

Bleiweis performed all kinds of work in different fields of significant importance, both in the professional and general sense. Therefore, it is rather difficult to summarize everything in one thought only. If we are to avoid enumeration and content ourselves with a simplification, we could say that Bleiweis strived to promote progress. He found various ways of working for the good of the Slovenian language and of the Slovenian community. If we summarize his efforts focusing on the Slovenian literary language, general use of the Slovenian language and the establishment of contacts among Slovenian, promotion of culture, knowledge and prudent management, as well as economic progress based on the nation's own capabilities, we obtain the framework of the programme which could be called the Slovenian national project. He was acknowledged as the leader of the Slovenian cause and served as an example, was even a charismatic figure: the father of the Slovenian nation.

In the 20th century, Bleiweis's work had been strongly criticised, whereby his true image and his work were distorted. Many a time these criticism were harsh, not at all consistent with his endeavours and his achievements, we could say even unjust.

SUBJECT INDEX VOLUME 45, 2008

7TM receptors 89, 102 ambient temperatures 127 analiti 133 analytes 127 arabian mare 25 arabske kobile 32 biokmarkerji 14 bivoli 60 borelijski antigeni 116 Borrelia antigens 109 Borrelia burgdorferi 15, 24 Bosnia and Herzegovina 43, 48 brejost 32 broiler 61, 127 brojlerji 66, 133 brown hare 33 buffaloes 53 calves 53 canine parvovirosis 73 Capybara 135 colloids 83 crystalloids 83 dairy cattle 15 dilatacijska kardiomiopatija 14 dilated cardiomyopathy 5 dimerizacija 102 dimerization 89 Doberman pinscher 5 dobermani 14 dogs 5, 73 drobnica 72 drought 121 echocardiography 5 eksperimentalni pristopi 102 esencialno olje - origano 66 estradiol-17ß 25, 32 estrus 32 experimental methods 89 fluid therapy 83 FoxA3 103, 108 gastrin 121, 126 genetics 5 genetika 14 gestagens 25, 32

gestation 25 goat 67 govedo 24 haemonchosis 121, 126 haemorrhagic shock 83 helminth 67, 72 hemoragični šok 88 holter monitoring 14 hormones 127, 133 Hydrochaeris hydrochaeris 135, 140 IgG 53, 60 immunoenzime assav 109 immunohistochemistry 103 imunoencimske analize 116 imunohistokemija 108 infertility 103 koloidi 88 kristaloidi 88 kunec 42 lipid oxidation 61 m. cremaster 33, 42 mATPase 33, 42 miozin 42 modo 108 myosin 33 neplodnost 108 oestrus 25 oksidacija maščob 66 oregano essential oils 61 ovce 126 parasites 67 parvoviral enteritis 73 parvoviroza 78 parvovirusni enteritis 78 pathology 73, 135 patologija 78, 140 pepsinogen 121, 126 poljski zajec 42 praživali 72 predporodno obdobje 60 prepartum 49 prevalence 67, 72 priogranulomatozno vnetje 140 prirastek 66

protozoa 67 psi 78 pyogranulomatous lesions 135 rabbit 33 sera 127 serological diagnosis 15 serološka diagnostika 24 serum 133 sheep 67, 121 skeletal muscle fibres 33 skeletna mišična vlakna 42 species diversity 67 structure 89 struktura 102 suša 126 tekočinska terapija 88

teleta 60 temperatura okolja 133 testes 103 ultra-corn 53, 60 ultrasonography 25 ultrazvok 14, 32 urin 109, 116 veterinarska medicina 78 veterinarska služba 48 veterinary medicine 73 veterinary service 43 viability 53 vitalnost 60 viteselen 53, 60 Yersinia pseudotuberculosis 135, 140 zajedavci 72

AUTHOR INDEX VOLUME 45, 2008

Amer HA, Hashem MA. Reproductive
performance and viability of newborns in
buffaloes treated antepartum with viteselen
and/or ultra-corn
Amer HA, Shawki G, Ismail R. Profile of
steroid hormones during oestrus and early
pregnancy in Arabian mares
Bavdek SV. Some reflections on the figure
and work of the father of the Slovene nation 143
Bavdek SV. The work of Dr Janez Bleiweis
and his personality 144
Borko Č. Humoral immune response to
bacteria Borrelia Burgdorferi s.l. in dairy
cattle after experimental exposure 15
Burnik J. Forensic medicine – an unknown
side of Dr Janez Bleiweis 143
Cabadaj R, see Marcinčák S, Cabadaj
R, Popelka P, Šoltýsová L 61
Čeh K, Zorn B, Šinkovec J, Majdič G.
Imparied expression of FoxA3 is not a
common cause of sertoli cell-only syndrome
in human infertile patients 103
Cornwell SM, see Fejzic N, Šerić Haračić
S, Dargatz DA, McCluskey BJ, Cornwell SM,
Salman M, Mumford EL 43
Dargatz DA, see Fejzic N, Šerić Haračić S,
Dargatz DA, McCluskey BJ, Cornwell SM,
Salman M, Mumford EL 43
Domanjko Petrič A, Tomsič K. Diagnostic
methods of cardiomyopathy in dogs - old and
new perspectives and methods 5
Fazarinc G,see Štrbenc M, Fazarinc G,
Pogačnik A 33
Fejzic N, Šerić Haračić S, Dargatz DA,
McCluskey BJ, Cornwell SM, Salman M,
Mumford EL. Development of an animal
health surveillance infrastructure in
Bosnia and Herzegovina – case report
Gahlot AK, see Kataria N, Kataria AK,
Gahlot AK 121
Gahlot AK, see Kataria N, Kataria AK,
Gahlot AK 125
Gombač M, Švara T, Tadić M, Pogačnik M.
Retrospective study of canine parvovirosis in
Slovenia

Gombač M, Švara T, Zdovc I, Juntes P, Pogačnik M. Yersiniosis in captive capybaras (Hydrochaeris hydrochaeris) 135 Gruntar I, Malovrh T. Development and evaluation of monoclonal antibody-based enzyme linked immunosorbent assay for Borrelia garinii antigen detection in urine 109 Hashem MA, see Amer HA, Hashem MA..... 53 Islam KBMS, Taimur MJFA. Helminthic and protozoan internal parasitic infections in free ranging small ruminants of Bangladesh.... 67 Ismail R. see Amer HA. Shawki G. Ismail R... 25 Juntes P. see Gombač M. Švara T. Zdovc I. Juntes P, Pogačnik M 135 Jurca J. Dr Janez Bleiweis, his contribution to the evolution of the Slovene professional terminology 144 Jurca J. Janez Bleiweis and cattle plague ... 144 Kataria AK, see Kataria N, Kataria AK, Kataria AK, see Kataria N, Kataria AK, Kataria N, Kataria AK, Gahlot AK. Ambient temperature associated variations in serum hormones and interrelated analytes of broiler Kataria N, Kataria AK, Gahlot AK. Use of plasma gastrin and pepsinogen levels as diagnostic markers of abomasal dysfunction in Marwari sheep of arid tract 121 Kubale V, Pogačnik A, Schwartz TW, Vrecl M. Seven transmembrane receptors (7TM) in the view of dimerization and experimental methods to study their dimerization and cross-talk...... 89 Majdič G, see Čeh K, Zorn B, Šinkovec J, Majdič G 103 Majdic G. Editorial 141 Malovrh T, see Gruntar I, Malovrh T. 109 Marcinčák S, Cabadaj R, Popelka P, Šoltýsová L. Antioxidative effect of oregano supplemented to broilers on oxidative stability McCluskey BJ, see Fejzic N, Šerić Haračić S, Dargatz DA, McCluskey BJ, Cornwell SM, Salman M, Mumford EL..... 43

Mumford EL, see Fejzic N, Šerić Haračić S,
Dargatz DA, McCluskey BJ, Cornwell SM,
Salman M, Mumford EL
Osterc J. Bleiweis – a proponent of
stockbreeding
Pengov A. Medical and veterinary education
in the Austro-Hungarian monarchy up to the
19th century
Pogačnik A, see Kubale V, Pogačnik A,
Schwartz TW, Vrecl M
Pogačnik A, see Štrbenc M, Fazarinc G,
Pogačnik A
Pogačnik M, see Gombač M, Švara T, Tadić M,
Pogačnik M73
Pogačnik M,see Gombač M, Švara T, Zdovc I,
Juntes P, Pogačnik M 135
Popelka P, see Marcinčák S, Cabadaj R,
Popelka P, Šoltýsová L 61
Salman M, see Fejzic N, Šerić Haračić S,
Dargatz DA, McCluskey BJ, Cornwell SM,
Salman M, Mumford EL
Schwartz TW, see Kubale V, Pogačnik A,
Schwartz TW, Vrecl M
Seliškar A. Fluid therapy in haemorrhagic
shock
Šerić Haračić S, see Fejzic N, Šerić Haračić S,
Dargatz DA, McCluskey BJ, Cornwell SM,
Salman M, Mumford EL

Shawki G, see Amer HA, Shawki G,
Ismail R
Šinkovec J, see Čeh K, Zorn B, Šinkovec J,
Majdič G 103
Šoltýsová L, see Marcinčák S, Cabadaj R,
Popelka P, Šoltýsová L 61
Štrbenc M, Fazarinc G, Pogačnik A. Some
morphological aspects of the cremaster muscle
in brown hare and domestic rabbit
Švara T, see Gombač M, Švara T, Tadić M,
Pogačnik M73
Švara T, see Gombač M, Švara T, Zdovc I,
Juntes P, Pogačnik M 135
Tadić M, see Gombač M, Švara T, Tadić M,
Pogačnik M 73
Taimur MJFA, see Islam KBMS,
Taimur MJFA67
Tomsič K, see Domanjko Petrič A, Tomsič K 5
Vodopivec P. Janez Bleiweis – on the 200th
anniversary of his birth 142
Vrecl M, see Kubale V, Pogačnik A,
Schwartz TW, Vrecl M
Zdovc I, see Gombač M, Švara T, Zdovc I,
Juntes P, Pogačnik M 135
Zorn B, see Čeh K, Zorn B, Šinkovec J,
Majdič G 103

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Znanstveni članki in tisti, ki so prikaz lastnih raziskav in dognanj, morajo vsebovati še naslednje obvezne rubrike, s katerimi avtor sam naslovi ustrezne dele besedila v prispevku: Introduction, Material and methods, Results, Discussion in References. Pregledni članki naj vsebujejo uvod, poglavja, ki so glede na vsebino smiselno naslovljena, in literaturo. Podatke o financerjih ali drugih zadevah, pomembnih za prispevek, npr. o tehnični pomoči, avtorji navedejo v rubriki Acknowledgements, ki se uvrsti pred rubriko References. Za rubriko References sledijo spremna besedila k slikam.

Priloge, kot so tabele, grafikoni in diagrami naj bodo smiselno vključene v besedilo. Slikovni material naj bo poslan posebej v obliki bmp, jpg, ali tif.

Priloge in slike morajo biti poimenovane z besedami, ki jih opredeljujejo, in arabskimi številkami (npr. Table 1:, Figure 1: itn.). Za dvopičjem sledi besedilo oziroma naslov. Vsi navedki (reference), citirani v besedilu, se morajo nanašati na seznam literature. V besedilu jih je treba oštevilčiti po vrstnem redu, po katerem se pojavljajo, z arabskimi številkami v oklepaju. Prvi navedek v besedilu opredeli številko oziroma vrstni red ustreznega vira v seznamu literature. Če se avtor v besedilu pri prvem navedku. Citirana so lahko le dela, ki so tiskana ali kako drugače razmnožena in dostopna javnosti. Neobjavljeni podatki, neobjavljena predavanja, osebna sporočila in podobno naj bodo omenjeni v navedkih ali opombah na koncu tiste strani, kjer so navedeni. V seznamu literature so viri urejeni po vrstnem redu. Če je citirani vir napisalo šest ali manj avtorjev, je treba navesti vse; pri sedmih ali več avtorjih se navedejo prvi trije in doda et al.

Da bi se morebitni popravki lahko objavili v naslednji številki, jih morajo avtorji pravočasno sporočiti glavnemu uredniku.

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Slov Vet Res 2008; 45 (4)

Original nesearch rapers	
Kataria N, Kataria AK, Gahlot AK. Use of plasma gastrin and pepsinogen levels as diagnostic markers of abomasal dysfunction in Marwari sheep of arid tract	21
Kataria N, Kataria AK, Gahlot AK. Ambient temperature associated variations in serum hormones and interrelated analytes of broiler chikens in arid tract	27
Case Report	
Gombač M, Švara T, Zdovc I, Juntes P, Pogačnik M. Yersiniosis in captive capybaras (<i>Hydrochaeris hydrochaeris</i>)	35
On the occasion of 200 th anniversary of the birth of Dr Janez Bleiweis	
Editorial (Majdič G)	41
Summaries of Supplement 12 (Bavdek SV, Vodopivec P, Burnik J, Pengov A, Jurca J, Osterc J)	42
Subject Index Volume 45, 2008	47
Author Index Volume 45, 2008	49