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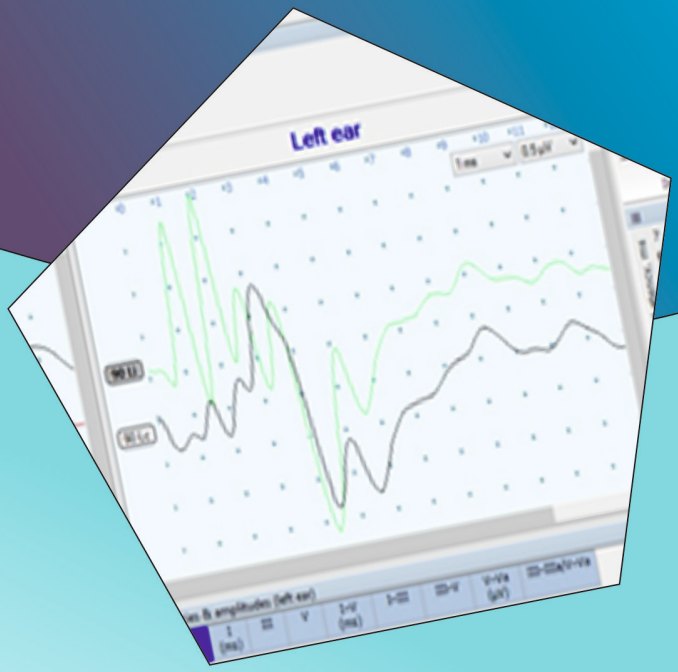

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Article

Evaluation of blood electrolyte alterations in cats during elective laparoscopic ovariectomy

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Abstract: In a clinical setting, we tested the hypothesis of whether hypercapnia developed during carbon dioxide pneumoperitoneum is associated with changes in blood electrolytes. This prospective study involved ten female cats that underwent elective laparoscopic ovariectomy. Venous blood samples for assessment of electrolytes were collected in the following sequence: T1- before anaesthesia induction, T2 - 10 minutes after anaesthesia induction, T3 - 30 minutes of pneumoperitoneum and T4 - at the end of pneumoperitoneum. Statistical analysis revealed AB disturbances associated with general anaesthesia and pneumoperitoneum, manifested with decreased blood pH, whereas blood $PvCO_2$, PO_2 and BE were increased. A constant increase of K+ concentration was recorded in all animals during pneumoperitoneum ($P<0.05$), whereas iMg registered a significant increase only at T3 ($P<0.05$). Correlations were recorded between blood pH and Na+, iCa, iMg, as well as between Na+ and Cl^- at different time points during anaesthesia. No correlations were noted between pH and K+ or $PvCO_2$ and K+. In conclusion, electrolyte imbalance represents a possible complication associated with laparoscopic surgery in healthy cats. However, further studies should investigate the causes involved in K+ concentration elevation.

Keywords: hyperkalemia, pneumoperitoneum, laparoscopy, feline

1. Introduction

Ovariectomy is the most common abdominal laparoscopic surgical procedure performed in small animals, which has gained popularity during the last decade [3]. The essential characteristic of laparoscopic surgery is represented by carbon dioxide (CO_2) insufflation inside the peritoneal cavity, to achieve surgical view and working space [8]. It has been postulated that these procedures are associated with lower morbidity, expedited recovery time and reduced postoperative pain. However, the pneumoperitoneum leads to increased abdominal pressure, which can further develop significant cardiovascular and respiratory changes, which represent a potential life-threatening condition [27][15].

Acid, base and electrolyte alterations are typically observed in the perioperative period, and are related to underlying conditions but may be also iatrogenic. General anaesthetics, intraoperative excessive tissue handling, the nature of fluids infused and metabolic derangements, have all been demonstrated to possess risks of inducing severe electrolyte imbalance [20]. In animal studies, laparoscopic surgery has been associated with alterations of acid-base (AB) metabolism as a manifestation of respiratory acidosis. Gas insufflation into the abdominal cavity highly affects respiratory mechanics through a reduced lung volume due to diaphragmatic limited excursions, diminished functional residual capacity and respiratory compliance [15]. Altogether, these factors can affect

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the lung compensatory mechanism of AB balance regulation, resulting in hypercapnia [22]. Moreover, the diffusion of peritoneal CO_2 into the bloodstream increases further the concentration of CO_2 ($PaCO_2$). This increase will ultimately lead to blood pH changes toward acidosis [28].

For optimal functioning of the cells, relative constancy of the body's pH is essential because metabolism requires enzymes that operate at fairly narrow limits of pH. Electrolytes represent cofactors in enzymatically maintained metabolic reactions, playing vital roles in cellular function, tissue perfusion and AB balance [22]. Any changes of pH could disrupt cell metabolism and consequently body function.

Several studies showing the cardiopulmonary changes due to CO_2 induced pneumoperitoneum have been reported previously in domestic cats, but such data regarding electrolytes homeostasis is lacking from the literature. Therefore, the objective of this study was to analyse selected electrolyte alterations in healthy feline patients undergoing laparoscopic ovariectomy, using an in-house blood gas analyser. Based on our current understandings, it was hypothesized that AB imbalance developed during laparoscopic surgery, even for a short period, can affect electrolyte balance.

2. Materials and Methods

In this prospective study, cats were enrolled for elective laparoscopic ovariectomy. Exclusion criteria included evidence of pregnancy and other abnormalities found on the physical examination. Surgical procedures were realized with the owner consent and the study was approved by the Comity for Bioethics and Research Ethics of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (aut. No 211 from 27.05.2020). All video-assisted ovariectomies were performed by the same surgeon.

Food was withheld one night before surgery. The anaesthesia protocol for each feline patient consisted of premedication with 20 mcg/kg of Buprenorphine (Buprecare 0.3 mg/ml, Recipharm Monts, UK) and 10 mcg/kg of Medetomidine (Domitor 1 mg/kg, Orion Corporation, Finland) by intramuscular administration. General anaesthesia was achieved by intravenous administration of 0.25 mg/kg Diazepam (Diazepam 5 mg/ml, Sun Pharma Company, Romania) and 3 mg/kg Ketamine (Narkamon Bio 100 mg/ml, Bioveta, Czech Republic), and maintained with 1.5% Isoflurane (Isoflutek 1000mg/g, Laboratorios Karizoo, Spain) in 100% Oxygen (1 L/min), using a rebreathing anaesthesia circuit (Dräger Medical, Germany). All animals received mechanical ventilation with a Dräger Fabius Plus XL anaesthetic machine (Dräger, Germany). Ventilator parameters were adjusted to deliver a tidal volume of 8-10 ml/kg at a respiratory rate to maintain an end-tidal carbon dioxide tension (EtCO2) below 60 mmHg. Fluid therapy with NaCl 0.9 % solution (NaCl 0.9% B Braun, Germany) was administered at a rate of 5 ml/kg/hour. A bolus of 6% hydroxyethylstarch (6% Hemohes 200/0.5, B Braun, Germany) of 2 ml/kg was administered over 2 min for patients with mean arterial pressure (MAP)<60 mmHg. Upon completion of the procedure, the animals were placed in a recovery box. Meloxicam (Melovem 5mg/ml, Dopharma, Romania) 0.2 mg/kg subcutaneously was administered for complete analgesia.

Monitoring of anaesthesia during the study was performed by use of a multiparameter monitor (Vista 120, Dräger, Germany) and included electrocardiography (ECG) using a Lead II configuration, puls-oxymetry (SpO_2), body temperature and non-invasive blood pressure. A small volume of approximately 1 ml of the venous blood sample was collected using a 2 ml pre-heparinized syringe (Luer Slip Blood Gas Sampling system, Numbrecht Germany) from the jugular vein and analysed using a point-of-care blood gas analyser (Stat Profile Prime Plus® VET Critical Care Analyzer, Nova Biomedical). The blood was evaluated for pH, $PvCO_2$ (partial pressure of carbon dioxide), PO_2 (partial pressure of oxygen), blood concentrations of sodium (Na^+), potassium (K^+), ionized calcium (iCa), ionized magnesium (iMg), chloride (Cl^-), glucose (Glu), base excess (BE), haematocrit (Ht) and haemoglobin (Hb), all parameters being integrated into the analyser panel. The measurements were performed after premedication (T1, baseline values), 10 minutes after induction of anaesthesia (T2, control), 30 minutes after the start of pneumoperitoneum (T3) and at the end of pneumoperitoneum (T4). A standard surgical procedure using a two-port approach was performed by the same surgeon. Insufflation pressure of CO_2 was limited to 6mmHg, consistent with previous clinical reports [25]

3. Statistical analysis

All data are reported as the mean ± SD. To assume Gaussian distribution normality distribution was checked by Shapiro-Wilk normally test. One-way analysis of variance ANOVA, followed by post hoc Tukey's range test procedure was done for pair-wise comparisons and Pearson test analyzed the correlation between normally distributed values. Pearson's correlation was used to assess the correlation between normally distributed variables; the interpretation was done according to Colton scale. Statistical significance was at $p<0.05$ (95% confidence interval). Statistical values and figures were obtained using GraphPad Prism version 5.0 for Windows, GraphPad Software, San Diego California USA.*3.1. Subsection*

4. Results

Measurement of electrolytes was successfully performed in ten healthy cats, American Society of Anaesthesiologists status 1, with a mean age of 14.4 months (range 6-48 months, Standard deviation (SD) ±12.92) and body weight 2.64 kg (2.2-3.3 kg ±0.35). All animals recovered uneventfully. Temporal changes were examined by comparing data with baseline and control values. Total mean anaesthesia time was 116.17 ± 43.72 min (range 80-210 min) of which pneumoperitoneum was 67.89 ±18.89 min (56-119 min). Premedication induced emesis in one cat. Rescue analgesia was necessary for 6 animals. Four animals required one bolus of 6% HES for hypotension correction (MAP <60 mmHg).

The hemodynamic variables and body temperature (BT) are listed in Table 1. There was no difference in MAP over the study. The heart rate (HR) showed an increase after the beginning of pneumoperitoneum ($P<0.05$; Table 1), and the BT was lower at T2, T3 and T4 compared with baseline values.

Table 1. Values of physiologic parameters in cats during laparoscopy surgery

Parameter	**T1** **Mean ± SD** **(Range)**	**T2** **Mean ± SD** **(Range)**	**T3** **Mean ± SD** **(Range)**	**T4** **Mean ± SD** **(Range)**
HR (bpm)	126 ± 10.96 (105-40)	119.89 ± 10.77 (107-138)	137.22 ±18.4 (98-167)	133 ±19.55 (107-162)
RR (breaths)	22.78 ±10.96 (13-34)	15.89 ±3 (12-22)	17.4 ± 2.22 (14-20)	17.56 ± 4.81 (12-27)
MAP (mmHg)	-	75.11 ± 19.06 (55-112)	82.33 ± 12.65 (65-110)	74.78 ± 11.96 (65-105)
BT (^{0}C)	38.56 ± 0.54 (37.6-39.3)	37.46 ± 0.85 (35.9-39)	36.96 ± 0.24 (36.5-37.2)	37.66 ± 0.65 (36.9-38.6)
SpO2 (%)		98.5 ± 1.81 (94-100)	98 ± 1.06 (97-100)	98.7 ± 1.21 (96-100)

HR indicates heart rate; bpm, beats per minute; RR, respiratory rate; MAP, mean arterial pressure; BT, body temperature; SpO2, oxygen saturation; SD, standard deviation.

The results of mean K+ concentration indicated a significant time effect, suggesting K+ levels change in time. During the anaesthesia, K+ increased from 3.75±0.56 mmol/L at T1 to 5.86±0.98 mmol/L at T4. Ionized magnesium concentration did not have a significant alteration, except at T3, which was considered higher than the value of T1. There were no significant changes in other electrolytes. Electrolyte trends with time are presented graphically in Fig.1.

The AB values showed variations in pneumoperitoneum patients as regard to blood pH which was significantly lower at T3 (P<0.05) compared with T1 and T2, increasing again by the end of surgery (Fig.2). PvCO2 and PO2 were almost two-fold increased at T2 and T3 as compared to T1 (P<0.05), while in T4 their level exhibited a decreasing trend. Mean BE level registered a significant elevation after induction of anaesthesia (P <0.05), this trend persisting throughout the surgery. Negative correlations were registered between pH and Na (P<0.05; Pearson's coefficient -0.74), iCa (P<0.001, Pearson's coefficient -0.95) and iMg (P<0.01, Pearson's coefficient -0.86) at T3. There was no significant correlation between PvCO2 and K+ or pH and K+ in any time point during pneumoperitoneum. No correlation was identified between surgical time and K+ level either.

As regard the blood glucose level, a significant increase occurred at T2 (p<0.05), persisting beyond baseline values until the end of surgery (Fig.2).

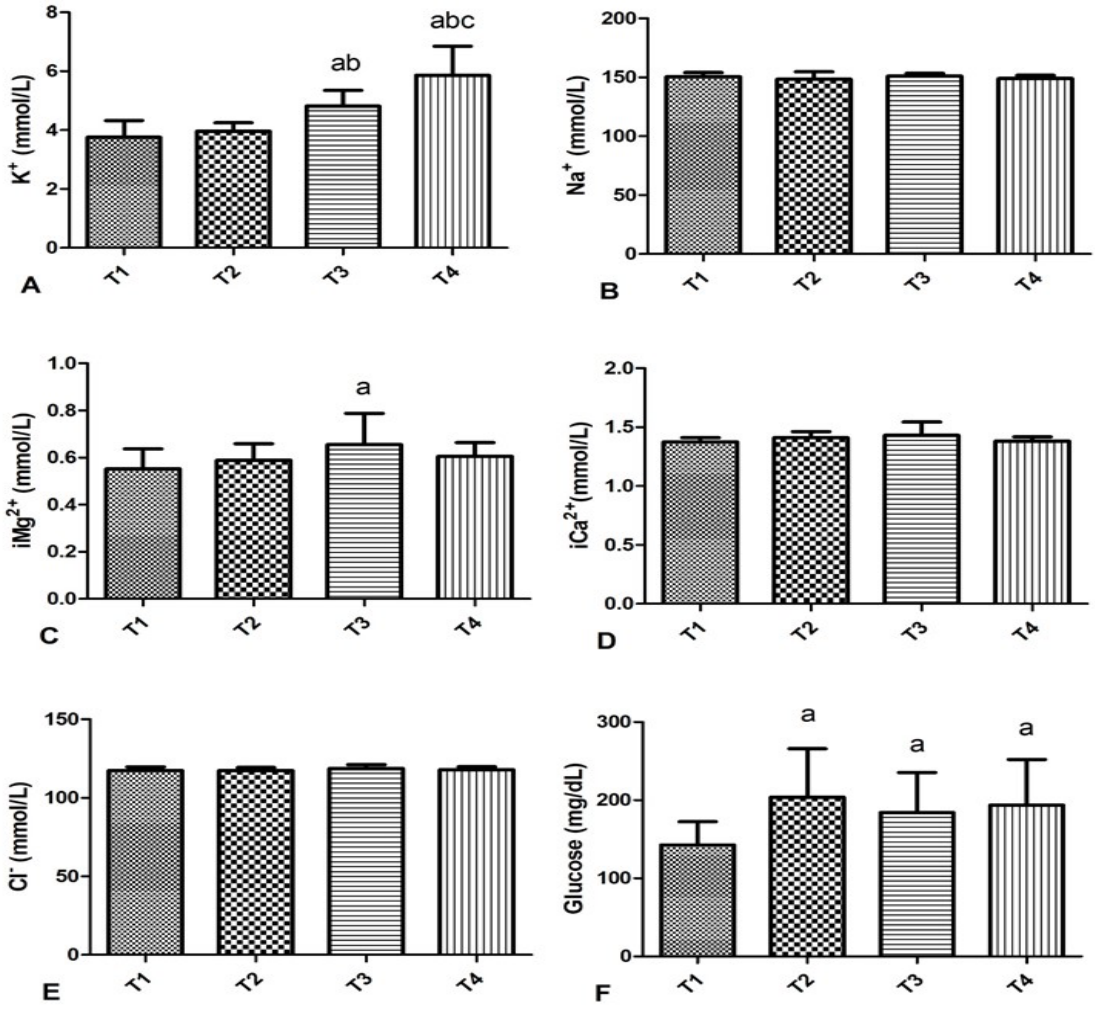

Figure 1. Effect of laparoscopic ovariectomy on blood electrolytes and glucose level. A – Potassium evolution, B – Sodium evolution, C – Ionised magnesium evolution, D – Ionised calcium evolution, E – Chloride evolution, F – Glucose evolution. (mean ± SD) (n = 9) (a = p<0.05 as compared to T1) (T1= Pre-induction, T2= control, T3= 30 min of pneumoperitoneum, T4= the end of pneumoperitoneum)

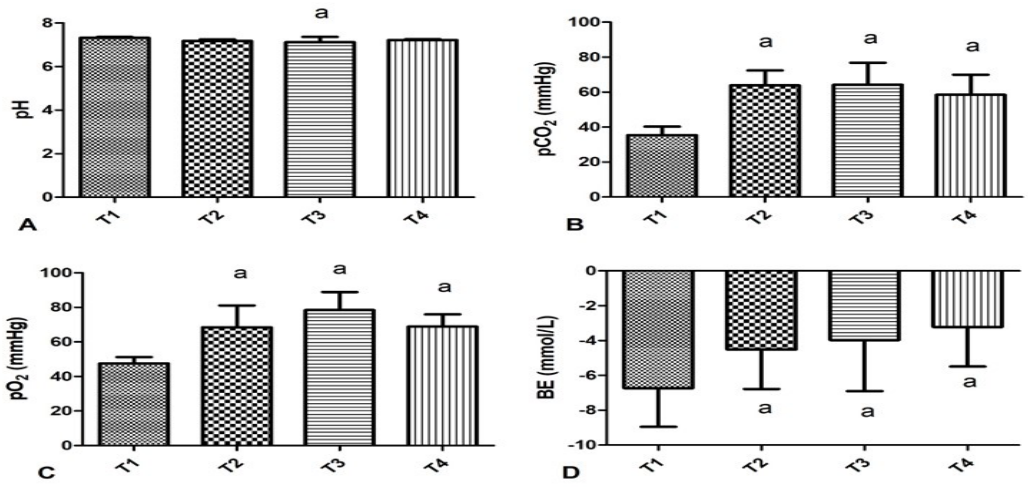

Figure 2. Effects of laparoscopic ovariectomy on blood AB values. A – pH evolution, B – PvCO2 evolution, C – PO2 evolution, D – BE evolution. (mean ± SD) (n = 9) (a = $p<0.05$ as compared to T1) (T1 = Pre-induction, T2 = control, T3= 30 min of pneumoperitoneum, T4= the end of pneumoperitoneum)

4. Discussion

Pressure insufflation of CO_2 inside the peritoneal cavity for laparoscopic surgery leads to undesirable conditions by causing modifications in respiratory, hemodynamic and AB balance [15]. In this study, changes in electrolytes in cats undergoing elective laparoscopic ovariectomy were evaluated. Our data suggests that some significant changes, although mostly transient, are evident. However, none of the changes of these parameters was of a magnitude likely to have clinical importance, at least not in healthy cats.

Blood analysis was performed from jugular blood collected by through venipuncture. Studies on feline AB values reported significant differences between venous and arterial samples [28], showing that arterial blood is indispensable for evaluating AB status, oxygenations and ventilation, but not for electrolyte imbalances. The purpose of the present report was to analyse the evolution of selected electrolytes in anesthetized cats; therefore, venous blood was preferred over arterial.

The most important electrolyte modification observed was the progressive increase in K+ concentration. These findings corroborate with those of previous studies evaluating the effect of CO_2 peritoneal insufflation effect on K+ homeostasis. Pearson et al. showed that following prolong insufflation of CO_2 into the peritoneal cavity in pigs, the K+ concentration increased over time [16]. Similar findings were registered by Demiroluk et al. and Singh et al. in human patients undergoing laparoscopic abdominal interventions [5] [24]. Likewise, Al-Badrany et al, in the experimental induced pneumoperitoneum study on dogs, showed a positive correlation between K+ and abdominal pressure [1]. Reilly et al. while assessing biochemical and electrolyte alterations in captive tigers during laparoscopic ovariectomy, observed a similar increase over time in all animals, one tiger developing hyperkalaemia associated with ECG modifications [21]. The changes in potassium concentration in the current study were without clinical expression; nonetheless, these alterations might be relevant in critically ill feline patients at risk for hyperkalaemia. Concerning iCa concentration, the modifications partially corroborate findings from one previous study in dogs [1]. Ionized calcium concentration showed a tendency to increase in time, without a significant difference from baseline values. These results were contradictory with the study conducted by Reilly et al. on anaesthetized tigers in which the plasma calcium showed a significant decrease over the course of anaesthesia [21]. Likewise, Garg et al. related hypocalcaemia in a human patient during laparoscopic repair of a diaphragmatic hernia [10].

However, in their report, the iCa imbalance was attributed to fat tissue handling and necrosis, due to calcium binding to adipose tissue.

There were no changes registered in Cl^- and Na+ concentrations in the present study. One study reported a change towards hyponatremia in dogs, but without significance, in response to hemodynamic instability [1]. As regard to iMg level, the findings were compatible with the study carried out by Kohler et al. in which was demonstrated that mobilization of magnesium from body tissues takes place if body pH is altered [14].

In our current investigation, the factors influencing the electrolyte balance varied during the anaesthesia. In the literature, modifications of blood pH and $PvCO_2$ have been suggested as causes to induce electrolyte imbalances [7], [22]. After induction of anaesthesia, there was an increase in the mean $PvCO_2$ concentration compared with baseline with significant value, attributable to anaesthesia drugs' specific respiratory depressive effects [11]. Once pneumoperitoneum started, there was a non-significant increase in the $PvCO_2$. During laparoscopy, hypercarbia results from CO_2 absorption through the peritoneal cavity into bloodstream and decreased alveolar ventilation [29]. As already demonstrated in previous studies, the increase in $PvCO_2$ was negatively correlated with a decrease in blood pH. Acidosis was a consistent finding during abdominal insufflation with CO_2. If we compare the means of venous blood pH at pre-induction and during the surgery, there were significant differences during pneumoperitoneum. In response to the acute respiratory acidosis developed, there was a significant difference at BE levels relative to that at the pre-induction time, suggesting a respiratory factor as the cause of the decrease of pH during the surgical intervention.

Clinical studies sustain a linear relationship between hypercapnia and hyperkalaemia [12]. During acidosis, the movement of K+ from intracellular space outwards occurs as a result of reduced Na+, K+-ATPase activity, with hydrogen entering in exchange of K+ [7]. Contrary to the aforementioned papers, the authors of this study could not identify any correlation between pH or $PvCO_2$ evolution and hyperkalaemia. These findings coincided with the report of Weinberg et al. on human patients with experimental induced hypercapnia [31]. In this context, it could be suggested that the significant hyperkalaemia in this trial reflects a combination of the effects of extracellular K+ shift due to acidosis and insulin deficiency and an assumptive decrease in renal perfusion. Administration of medetomidine in the present study caused a significant increase in glucose level in all patients, as a consequence of its mechanism of action by insulin inhibition [13]. Insulin deficiency plays an important role in the net efflux of K+ from the cell, as frequently seen in diabetic patients [7]. In the study by Rilley et al. on electrolyte alterations in non-domestic felids, hypoinsulinemia coincided with hyperglycaemia and induced clinically important hyperkalaemia in one anesthetised tiger. This finding was in accordance with our results; however, hyperkalaemia has been shown to be a complication frequently associated with administration of alpha 2 adrenergic agonists in large felids [26]. Kidneys play an esential role in AB and electrolyte balance. Temporary reversible oliguria during pneumoperitoneum has been observed in clinical and experimental studies as a consequence of low renal perfusion [19]. This side effect could have been associated to a diminished K+ excretion; however, in our study, urine output was not monitored.

Pearson and Sander, in their experimental study performed in pigs, concluded that long duration pneumoperitoneum can lead to clinically important hyperkalemia [16]. In our study, the average time of pneumoperitoneum was 68 min, with a wide range of 56 and 119 min. There was a significant increase in K+ value at a peritoneal pressure maintained stable at 8 mmHg, although no relationship between the time of pneumoperitoneum and potassium concentration was found.

On the other hand, the iCa and iMg evolution were negatively correlated with pH evolution. Both calcium and magnesium can be found in circulation in different fractions, from which the ionized form usually reflects the true status in case of AB disturbances [2] [23]. Clinical and laboratory researches have already demonstrated the pH influence on iCa concentration, suggesting a magnitude of 0.05 mmol/L for every 0.1 pH change [17]. Similarly, in the study by Wang et al. (2002), pH proved to have a direct effect on magnesium measurement, as a result of a weak binding to plasma protein in a more acidic environment [30]. In the current study, this relationship was highlighted by the patient with severe respiratory acidosis seen at min 30 of pneumoperitoneum, in which iCa reached the highest value (1.72 mmol/L), at a level considered in

veterinary medicine as severe hypercalcemia [4], associated with a concomitant increment of iMg (0.98 mmol/L). The authors attributed this imbalance to improper ventilation, BG and electrolyte analysis improving after ventilator settings were modified.

A negative correlation was recognized at T2 between Cl^- and Na+ concentration. The correlation was changed during pneumoperitoneum, the variables moving together in a positive direction. These findings could be attributable to a renal and gastrointestinal response to acute respiratory acidosis. Ramadoss et al. demonstrated the renal response manifested through Cl^- excretion with respect to Na+ within 30 minutes of acute respiratory acidosis [18]. As a further compensation mechanism, as suggested by Feldman and Charney, gastrointestinal reabsorption of both electrolytes is initiated [9]. Nevertheless, additional research is needed to support this supposition.

There are limitations in our study. First, there were no assessments performed after recovery from anesthesia. In addition, a small number of animals was involved in the study and the absence of a control group. The use of control group undergoing open laparotomy procedure would have been of interest in comparatively monitoring electrolyte disturbances. Moreover, it would be recommended to further evaluate these findings with different anaesthesia protocols.

In summary, the present study demonstrates a significant rise in K+ and iMg concentrations, not sufficient enough to cause clinical expression, in healthy cats undergoing elective laparoscopic ovariectomy. These changes occurred partially as a consequence of decreased blood pH and hypercarbia, and were associated with general anaesthesia, CO_2 gas diffusion and increased intraperitoneal pressure. However, further studies should investigate other possible causes of K+ concentration increase, as this parameter had the most significant alterations. The possibility of hyperkalemia has to be considered in all patients undergoing laparoscopic surgery, possessing a real challenge to anaesthetists. Therefore, monitoring of electrolytes may be important for increased safety during laparoscopic surgeries, particularly in patients with risk factors.

Author Contributions: IM, CP, LB and CAD initiated the study and designed the experiments. IM and MD contributed with data collection. Data management was done by IM, CP and SB. Data analyses and preparation of was done primarily by SB, with contributions from IM. IM, CP and LB drafted the manuscript. LO critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Article

Epidemiological aspects, haematological and biochemical alterations in some gastrointestinal parasitic diseases of carnivores

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Abstract: Gastrointestinal parasites are widespread pathogenic agents and one of the main causes for mortality in young dogs and cats. Many of these zoonotic parasites are relevant in terms of public health. The presence of parasites in the animal organism causes local and general modifications in the various organs they parasitize or transit throughout their life cycle. The present study aimed to identify the most frequent gastrointestinal parasites of dogs and cats and to monitor the alterations that occur in terms of haematological and biochemical parameters. The studied animals, 25 dogs and three cats from Timiș and Caraș Severin counties, were brought to the On-call room of the University Clinics of the Faculty of Veterinary Medicine Timișoara. The laboratory methods that were used were the Willis flotation method, the Baerman larvoscopic method and the Lugol method. The haematological methods, namely flow cytometry, cytochemistry and spectrophotometry, were performed at Bioclinica Laboratories, on whole blood samples that were collected in EDTA or simple tubes. The studied animals were positive for *Giardia*, *Cystoisospora*, *Dipylidium*, *Ancylostoma*, *Toxocara* and *Trichocephalus*. The positivity rate was 57.14%, with prevalence rates according to the parasitic species ranging from 3.57% to 21.42%, with multiparasitism in 32.14%, and monoparasitism in 17.85%. The values recorded for red blood cells, haemoglobin and hematocrit followed the same trendmost of the animals being situated within physiological values, except for three dogs, that recorded values below the minimal level. In the case of MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) the values recorded for most dogs were within physiological limits, except for three dogs which overpassed the maximum level. Eosinophils were high in all dogs, which is a characteristic feature of parasitism. The serum urea concentrations revealed the fact that all for dogs that were taken into study had values above the maximum limit.

Keywords: dog; cat; gastrointestinal parasites; haematological and biochemical parameters.

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1. Introduction

Both dogs (*Canis lupus familiaris*), and cats (*Felis catus*) are domestic animals that have maintained tight bonds with humans and were bred and kept out of various reasons: as pets, as hunting dogs, police and utilitary animals, laboratory animals and in the case of cats, for rodent-control.

Intestinal parasites, including protozoa and helminths, are among the most widespread pathogenic agents encountered by veterinarians, being one of the main causes for mortality in young dogs and cats.

Dogs can serve as definitive host for a variety of macroparasites, microorganisms and viruses. They are associated with tens of zoonotic diseases which bear a negative

impact on human and animal health [1, 2]. The most important ones are rabies, echinococosis, toxacariasis, ancylostomiasis, giardiasis, etc.

Many of these zoonotic parasites are relevant in terms of public health. Their transmission occurs by means of parasitic elements such as eggs or larvae. The resistance of eggs in the environment is quite high. In temperate areas, eggs can survive from 6 to 12 months and the most commonly used disinfectants are not effective against them [3].

Gastrointestinal parasites have undergone a successful evolutionary process and have managed to survive and move with the use of various organ systems of hosts. The result of such infections can vary from subclinical to severe, life-threatening ones. They can cause serious health issues translated through delayed growth, precarious health conditions, low resistance to other concurrent diseases and decreased productivity [4].

The presence of parasites in the animal organism causes local and general modifications in the various organs they parasitize or transit throughout their life cycle. The present study aimed to identify the most frequent gastrointestinal parasites of dogs and cats and to monitor the alterations that occur in terms of haematological and biochemical parameters.

2. Materials and Methods

The studied animals were brought to the On-call room of the University Clinics of the Faculty of Veterinary Medicine "King Michael the Ist of Romania" from Timișoara, between April 2018 and April 2019. The representative species were 25 dogs and three cats from Timiș and Caraș Severin counties. There were both pure bred and mixed breed animals, such as: German shepherd, Bucovina shepherd, Doberman, pincher, Alaskan malamute, viszla, Caucasian shepherd, Siberian husky, teckel, bichon, pug, poodle, Romanian mioritic shepherd, golden retriver, Shar Pei, Swiss shepherd, Siberian cats, European cats. The age of the animals ranged between: 2 months and 9 years and they were brought in for coproparasitic exams, with owners accusing to have noticed clinical signs specific for parasitic diseases, namely soft stools.

The laboratory methods that were used were the Willis flotation method, the Baerman larvoscopic method and the Lugol method [5 ,6].

The haematological methods, namely flow cytometry, cytochemistry and spectrophotometry, were performed at Bioclinica Laboratories, on whole blood samples that were collected in EDTA or simple tubes.

3. Results

The results of the parasitological exams highlighted pathogens that are sysmetically classified as part of the *Protozoa, Cestoda* and *Nematoda* classes. Thus, we have identified the following genera *Giardia, Cystoisospora, Dipylidium, Ancylostoma, Toxocara* and *Trichocephalus*.

Out of 25 dogs, four mixed breed and 21 purebred dogs, 11 were negative from a parasitololgical point of view and 14 were positive. As for the cats, two were positive for parasites (European) and one was negative (pure breed).

The concurrent infection with more than one species of parasites was observed in nine animals out of 28 taken into study (32.14%), while monoparasitism was noticed in five animals (17.85%) (fig. 1). In relation to the total number of animals taken into study, positive results were noticed in 16 animals, representing 57.14%.

The prevalence according to the identified parasite species varied between 3.57% and 21.42%, as seen in figure 2.

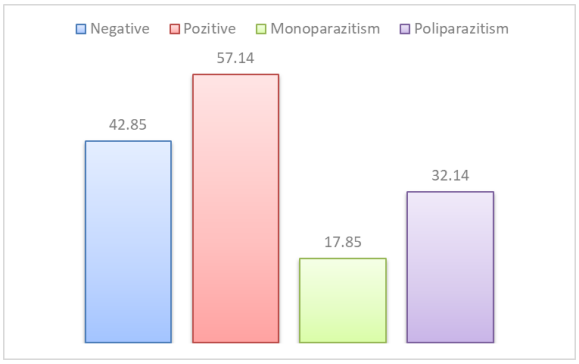

Figure. 1. Synthetic presentation of parasitism seen in the studied animals a parazitismului la animalele luate în studiu

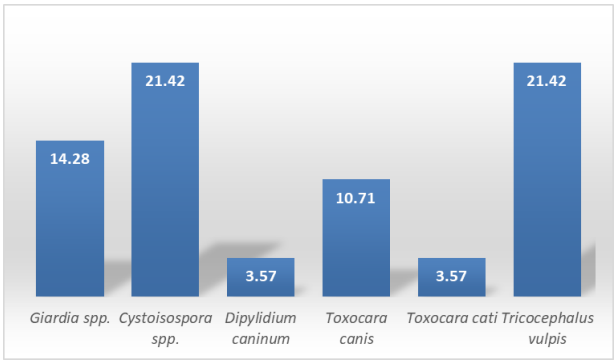

Figure. 2. Prevalence of the identified parasites in the studied animals

The haematological test values recorded for the 14 parasitized dogs are shown in figures 3-9.

The values recorded for red blood cells disclose the fact that the values from most dogs were within the minimal and maximal reference values, except for three of them that showed values below the minimal reference value. The values for red blood cells from one dog reached the maximal reference value.

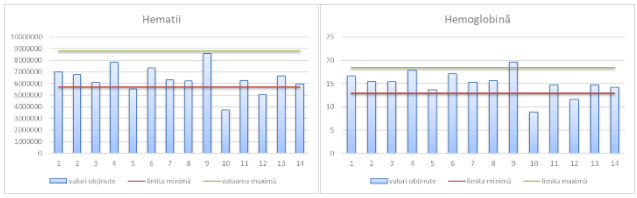

Fig. 3. Values recorded in the case of erythrocytes and haemoglobin

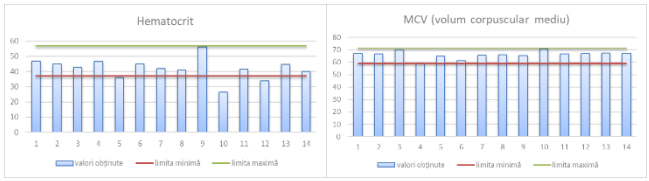

Fig. 4. Values recorded for the haematocrit and MCV

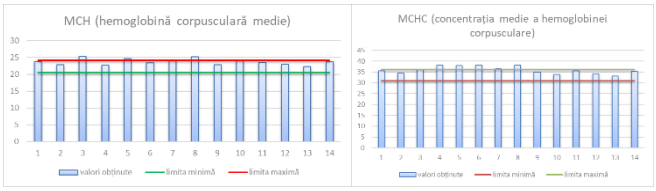

Fig. 5. Values recorded in the case of MCH and MCHC

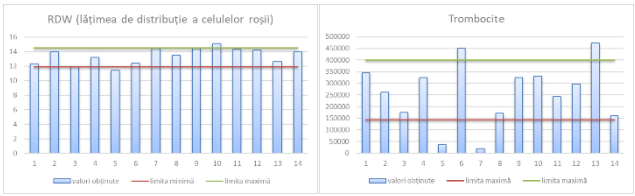

Fig. 6. Values recorded in the case of RDW and platelets

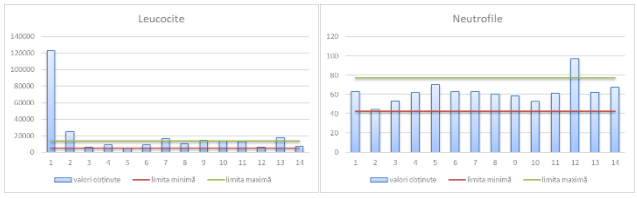

Fig. 7. Values recorded in the case of leukocytes and neutrophils

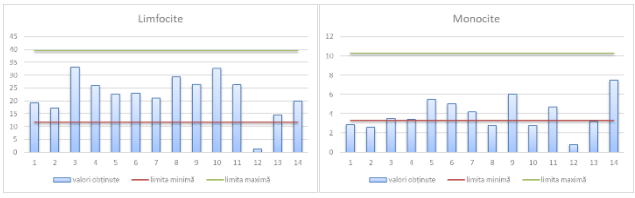

Fig. 8. Values recorded in the case of lymphocytes and monocytes

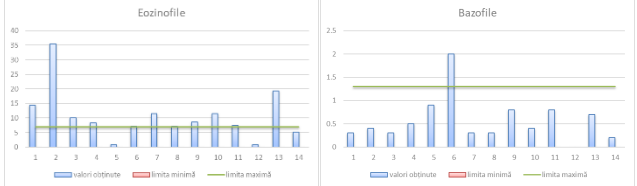

Fig. 9. Values recorded in the case of eosinophils and basophils

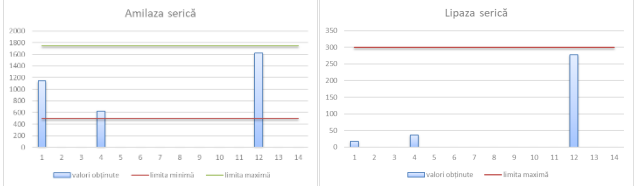

Fig. 10. Values recorded in the case of amilase and serum lipase

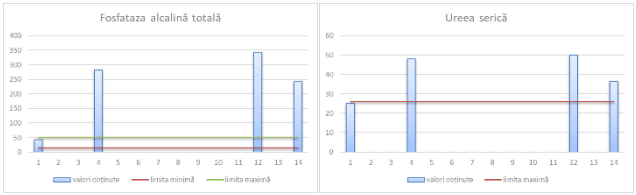

Fig. 11. Values recorded in the case of alkaline phosphatase and serum urea

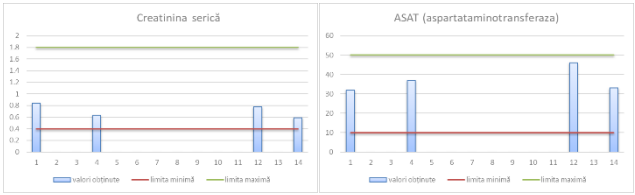

Fig. 12. Values recorded in the case of serum creatinin and ASAT

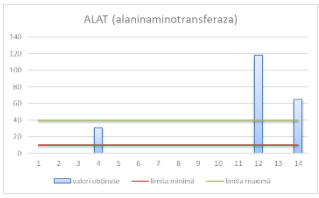

Fig. 13. Values recorded in the case of ALAT

In the case of haemoglobin, similar to the evolution of the red blood cells values, most dogs overpassed the minimal value, except for two of them that showed values below minimum. One of the dogs had values surpassing the maximal threshold. The study of the haematocrit revealed that the recorded data followed a similar trend as that seen in erythrocytes and haemoglobin.

In the case of MCV (mean corpuscular volume), all dogs presented values that were within the physiological limits, however two of them reached maximal values.

In what regards the MCH (mean corpuscular haemoglobin), all dogs showed values above minimum, three of them showed maximum values and three showed values that were above maximum.

All dogs had MCHC (mean corpuscular haemoglobin concentration) values higher than minimum, five dogs reached the maximum level and four of them overpassed it.

The study of the RDW (red cell distribution width) revealed the fact that most dogs from the study have surpassed the maximum value, except for one which showed values below the minimal threshold. Three of them reached the maximum value and one of them overpassed it.

In the case of platelets, the noticed values were above the minimal threshold for most cases. Two cases exhibited values below minimum and other two cases had values above the maximum threshold.

The leukocyte values did not go below average values for none of the cases, three of them reached the maximum limit, other four overpassed it with one of them overpassing the limit by far. This fact can be explained by the presence of a chronic infection or of an overlapping bacterial infection which happen at the same time with the migration of parasites throughout the host's body, in case of leukemia or in case of severe stress.

As for the neutrophils, all dogs showed values within normal ranges, with a single case showing values above maximum.

The lymphocyte values for most dogs were above the minimal limit, except for one which showed values below limit. None of them showed values above maximum level.

The monocyte values of the studied dogs were above the minimal limit, except for five of them. None of the cases showed values below minimum.

The eosinophils were above minimum for all dogs, except for three of them and one of them had very high values.

The basophil values did not overpass minimal values except for one of the dogs.

Biochemical analyses, in three, respectively four of the parasitized dogs, showed values that are presented in detail in figures 10-13.

Serum amylase values for the three studied dogs were all above minimum but below maximum.

Similarly, serum lipase was also below maximum levels.

Alkaline phosphatase in the case of the four studied dogs showed values above minimum, with three of them showing values above maximum. Following serum urea evaluation, it was revealed that all four studied dogs had values above the maximum reference level.

Serum creatinin for the four studied dogs revealed that all of the canine patients had values above minimum but below maximum.

AST varied similarly, with values above minimum but below maximum in all four dogs and ALT was evaluated in three dogs, all of them showing values above minimum, with two showing values above maximum as well.

4. Discussion

Mircean et al., 2012, identified the following parasites in dogs: *Toxocara canis* 26.9%, *Isospora ohioensis* 23.1%, *Ancylostoma caninum* 17.3%, *Uncinaria stenocephala* 13.5%, *Trichocephalus vulpis* 11.5%, *Hammondia heydorni/Neospora caninum* 9.6%, *Sarcocystis* spp. 9.6%, *Isospora canis* 7.7%, *Capillaria aerophila* 5.8%, *Strongyloides stercoralis* 93.8%, *Dipylidium caninum* 1.9% and *Toxascaris leonina* 1.9% [7].

In a study by Dărăbuș et al., 2018, eggs of *Toxocara canis*, *Ancylostoma caninum* and *Trichocephalus* spp. have been identified in varying proportions (0.57%, 5.78% and 1.73 – 2.22%, respectively) in dog faeces samples from parks.

Qadir et al., 2011 revealed a prevalence of 10.25% for mixed infections with *Ancylostoma caninum*, *Toxascaris* spp. and *Dipylidium caninum* and a 19.5% overall prevalence of different species of the helminths [8].

In Argentina, faecal samples from 85 dogs were examined for intestinal parasites. Seventeen parasite species were seen, of which 77% are zoonotic. The most prevalent parasites were *Ancylostoma caninum* (68.2%), *Giardia* spp. (25.9%), *Cryptosporidium* spp. (20.0%) and *Toxocara canis* (14.1%) [9].

Bishop and DeBess, 2020, in Portland, Oregon, United States of America, determined the following prevalences: *Toxocara canis* 8.7%, *Strongyloides* spp. 2.3%, *Trichuris* spp. 2.1%, hookworms (*Ancylostoma caninum*, *Uncinaria* sp., unspecified) 2.1%, *Cystoisospora* spp. 1.4%, *Taenia* spp. 1.2%, *Ancylostoma caninum* 0.9%, *Uncinaria* spp. 0.7%, *Baylisascaris procyonis* 0.2%, and *Dipylidium* spp. 0.2%, in canine fecal samples [10].

A study conducted by Regidor-Cerrillo et al., 2020, in Spain, highlighted that microscopic, gastrointestinal parasite forms found in dog faeces were identified as nematodes (*Ancylostomatidae*, *Toxocara canis*, *Trichuris* spp. and *Toxascaris leonina*), cestodes (*Taeniidae*) and protozoa (*Cystoisospora* spp. and *Giardia*). From the 233 analysed dogs, 63.5% were positive for at least one intestinal parasite, indicating a high degree of intestinal parasitism in these animals [2].

Significant decrease in the values of haemoglobin, total erythrocytic count, lymphocyte and marked leucocytosis, eosinophilia and neutrophilia were noted in pups that were naturally infected with toxocariosis. Post-treatment haematogical observations noted on day 7, 14 and 21 revealed that Toxocara infected dogs gradually returned to normal level but rapid and significant change towards normality were seen in Plozin (a combined tablet containing 500 mg fenbendazole, 144 mg pyrantel pamoate, 50 mg praziquantel @ 1/2 tablet per 5 kg body weight) treated animals [11].

Voßmann (1985) examined the haemtaological alterations in pups, following prenatal infections as well as the numeric changes of red blood cells [12]. The number of red blood cells in the blood stream is low from a physiological point of view in newborn pups, rising in approximately 6-8 weeks post partum to reach the values seen in adult dogs. In contrast with this fact are pups which are heavily infected with T. canis. They showed decreasing values of erythrocytes, mainly caused by severe internal bleedings. The reason behind these internal bleedings were the preadult larvae, that migrated through the liver and perforated the intestine, all aspects caused by the massive parasitic load. Pups with moderate infections showed an increase in the number of erythrocytes starting with the 5th week of life, but without reaching the values seen in non-infected animals. No changes in the numbers of red blood cells were noticed in adult bitches following infection with T. canis larvae [13]. Eosinophilia is characteristic in *T. canis* infections, starting from day 7 post infection and reaching maximum values within 14 days p.i. A similar evolution of eosinophilia is seen in pups infected in the prenatal period, starting with day 7 post partum [12]. Voßmann (1985) has also shown that the degree of eosinophilia in pups that were infected in the pre-natal period is almost proportional with the intensity of the infection [12]. Once the eggs start being shed in the faecal matters, the number of eosinophils slowly dropped, returning to physiological levels in 42 days p.i. These data have shown that the evolution of eosinophils in pre-natally infected pups is comparable to that of experimentally infected adult dogs [13].

Haematological changes are not the sole observations during infections with *T. canis*. Enzymatic alterations are also present thus, during the liver-migration period, the glutamate-dehydrogenase (GLDH) and alanintransaminase (ALT) rise, reaching maximum levels within 14 days p.i. [13]. After this peak, ALT stays at high levels for a certain period of time, while GLDH came back to normal values after 14 days [14].

Following reinfestation, hepatic enzymes show a new increase, although smaller in magnitude than during primary infection [13]. Voßmann (1985) noticed that these two enzymes, in pre-natally infected pups, were already high upon birth: 67 U / l for GLDH (reference values are up to 6,0 U / l) and 365 U / l for ALT (reference values are up to 55 U / l) in heavily infected pups [12]. Values came back to normal within 1-2 weeks post partum. A second increase in values caused by adult ascarids migrating through the liver and peritoneal cavity was detected shortly before the death of heavily infected pups.

The somatic migration in the lungs leads to multiple haemorrhagic petechia, forming a "tinted" pattern on the lungs [15]. The findings of Manhardt (1980) have confirmed that somatic migration is performed by larvae that are captured in the capillaries, larvae which penetrate their walls and migrate through the tissue in order to re-eneter the vascular system. This capacity to perform somatic migrations leads to the presence of larvae in organs founds in the vicinity of lungs and in the pleural cavity. Additionally, Manhardt (1980) has closely examined the kidney, an organ that is frequently affected by *T. canis*, noticing that severe organ failure is a rare sight in these situations. The larvae leave the blood vessels, namely the cortex, leaving small haemorrhages below the renal capsule, and begin a somatic migration. Some larvae go into the urinary canalicules, becoming detectable even in urine while others stay under the capsule after a short somatic migration and encapsulate in granulomas.

Shortly after beginning the hematogenous journey, larvae were also discovered in muscle fibers of the heart [15].

5. Conclusions

The carnivores that were taken into study were positive for pathogens from the *Protozoa*, *Cestoda* and *Nematoda* classes. The following genera were identified: *Giardia*, *Cystoisospora*, *Dipylidium*, *Ancylostoma*, *Toxocara* and*Trichocephalus*.

Compared to the toal number of examined animals, the positivity rate was 57.14%, with prevalence rates according to the parasitic species ranging from 3.57% to 21.42%, with multiparasitism in 32.14%, and monoparasitism in 17.85%.

The values recorded for red blood cells, haemoglobin and hematocrit followed the same trendmost of the animals being situated within physiological values, except for three dogs, that recorded values below the minimal level.

In the case of MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) the values recorded for most dogs were within physiological limits, except for three dogs which overpassed the maximum level.

Eosinophils were high in all dogs, which is a characteristic feature of parasitism.

The serum urea concentrations revealed the fact that all for dogs that were taken into study had values above the maximum limit.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, M.S.I. and R.G.O.; methodology, R.G.O; software, M.S.I.; validation, M.S.I. and R.G.O.; formal analysis, M.I, and I.L.; investigation, R.G.O, I.L. and S.G.; resources, T.S. and G.O; data curation, R.G.O. and G.O; writing—original draft preparation, M.S.I and T.S.; writing—review and editing, M.S.I and S.M.; visualization, T.S.; supervision, S.M.; All authors have read and agreed to the published version of the manuscript".

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Conflicts of Interest: The authors declare no conflict of interest.

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Case report

The influence of Electroacupuncture on a dog diagnosed with osteoarthritis: A case report

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Abstract: Electroacupuncture is a specific branch of acupuncture that uses electrical stimulation through the selected acupoints. Osteoarthritis is considered a complex condition associated with painful joints and locomotor dysfunction.

The aim of this case report was to bring scientific support regarding the effect of electroacupuncture in a dog with chronic joint degeneration.

An 11-year-old male German shepherd referred to the University of Agricultural Sciences and Veterinary Medicine of Cluj with severe pain in his hind limbs and around his back. Diagnosis based on a western examination, neurological assessment and radiographs indicated chronic osteoarthritis with hip dysplasia. From Traditional Chinese Veterinary Medicine based diagnosis was Kidney Qi Deficiency leading to Bony Bi Syndrome. For the last two years, the treated dog with Mavacoxib (single dose every month) did not show any significant improvement.

A combination of a fine needle with dry acupuncture, electroacupuncture (30-40Hz alternated with 80-100 Hz) and aqua-acupuncture using Zeel (AP) was performed. During the winter, weekly treatment was planned, after that, every two weeks treatment with electroacupuncture and dry needle, for five months until the present.

Since we started the acupuncture treatment, the dog is more active and enjoys playing again. We have managed to stop the administration of NSAID's and improve his life quality.

In the present study, we evaluated the effect of complementary medicine on a dog with chronic pain and joint degeneration. Electroacupuncture is a complex technique that requires special training; if used wisely, it can be an excellent complementary therapy for veterinary patients' pain control.

Keywords: dog; electroacupuncture; osteoarthritis; complementary medicine.

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1. Introduction

Osteoarthritis (OA), also known as a degenerative joint disease (DJD), is an inflammatory condition that occurs progressively in the joint as a result of loss of hyaline cartilage[1]. Cartilage is made of chondrocytes and is composed of type II collagen and a proteoglycan called aggrecan, which represents the link with hyaluronic acid[2]. In cases of OA, with the appearance of predisposing factors such as age, injury or various pathologies (obesity, poor conformation, prior elbow or hip dysplasia), the cartilage begins to decompose. This action leads from pain to immobility due to inflammation. It is most common in the lower limbs and in the lumbar-sacral area[3]. Large breed dogs, such as German Shepherd, Labrador or Golden Retrievers are more predisposed to this condition[3].

Traditional Chinese Veterinary Medicine (TCVM) has its origins in Old China and has been used for treating animals for thousands of years until present[4].

Meridians are represented by a complex system in which all the tissues and organs are connected and have an important role in the treatment of acupuncture[4]. The meridians transport the Qi and Blood through the whole body. This communication allows all the organs to coordinate and maintain an equilibrium of the system[4].

Acupuncture (Ac) is a complex method that uses very thin, filiform needles that are inserted in special points called "acupoints". Some recent studies show that these acupoints are actually the "locus" where nerves enter tissues or branches[5].

Electroacupuncture (EA) uses electric current (alternative, continuous or intermittent) which passes through needles that are already inserted into acupoints. EA is useful in case of degenerative lesions of the nervous system[5].

The aim of this study was to investigate the efficacy of EA and assess an acupuncture protocol for pain management in the case of OA. Also to reduce or, if possible, stop the administration of NSAIDs and improve quality of life.

2. Materials and Methods

2.1. Case description

Rufus is an 11 years old, male, German shepherd with chronic pain on his hind limbs, hip dysplasia and chronic keratitis. About 3 years ago, the dog started to have back pain around his hips and to crawl his hind limbs. Since then he was kept on NSAIDs (Mavacoxib – single dose every month) and Gabapentin when needed. From time to time, he loses stool and eats a smaller quantity of food, but after that, he starts eating better.

On October 2020, when M.F.D examined the dog for the first time. The mucous membranes were pink with a capillary refill of <2sec. The overall conformation was good with a 7/9 nutritional status. The coat was good, except for a small amount of hair loss. The dog was panting, but the respiration was unlabored. The body temperature was slightly elevated at 38.3°C. The pulse rate was 96 bpm. Neurological examination was within normal except for the presence of pain in the caudal area of the hips and proprioception was delayed on his right back leg.

From the TCVM point of view, the patient has an outward appearance as previously described. When observed, Rufus seems to be an Earth Constitution; he is friendly and communicative with people, but with other animals is aggressive, especially with cats. He lives with another German Shepherds, female and a Golden Retriever, male. He likes to be with people in the house, but he prefers to sleep outside. Rufus has a capricious appetite; he eats dry kibbles and cooked food (meatballs with different herbs). The dog prefers cold and seems better when he stays outside, in the yard. The tongue is slightly pale, thin with a white coat. The pulse was stronger on his left side. Sensitivity was noticed at Bladder (BL 17), Bladder (BL18), Bladder (BL 20), Bladder (BL22), Bladder (BL 23) and Bai-Hui. He did not let me touch his back.

From a Western diagnostic point of view, Rufus has hip dysplasia and osteoarthritis. From TCVM diagnostic point of view, Rufus has Bony Bi Syndrome (Kidney Yang and Qi Deficiency) and Blood Deficiency.

2.2. Treatment plan

A combination of a fine needle, dry acupuncture (Ac), electrical acupuncture (EA) at 80-100 Hz, aqua-acupuncture using (AP) was performed. Weekly treatment during the winter was planned. After that, every two weeks treatment with EA and Ac, until in present.

The treatments were performed using sterile disposable stainless-steel Ac needles with copper coil handle, size 0.25x25mm, guide tubes (Acimut), and stainless-steel Ac needles with plastic handle, size 0.22x25mm, without guide tubes (Cloud and Dragon) and sterile acupuncture needles 0.25x30mm with silver handle (Ener-Qi). Dry needling (insertion at 1cm-1.5cm with an intermittent manipulation of the point by twirling anti-clockwise) and EA (insertion at 1cm-1.5cm, 40Hz and ~1.5V-2V for the first 5-10 minutes, then 80-120Hz and ~1,5V-2V for 10-20 minutes) was used during the 15-20 minutes of each treatment, depending on the reaction of the dog. The acupoints used are presented in Table 1. We used Electro-Acupuncture Stimulator JM-3A by Dr Xie Huisheng (Figure 1). During each treatment, a maximum of six to eight points were used, depending on dog's reaction. The acupoints were selected according to TCVM principles. Every once a month we used Zeel injection solution (2.0 ml Ampules).

Table 1 Acupuncture points used

Local points	*Bai-Hui*, Shen-Shu, Shen-Peng, Shen-Jiao
Constitutional points	GV20, KID3, KID6, BL23, ST36, GB29, GB30, BL54
Association Points	BL11, BL18, BL20

Figure 1. Electro-Acupuncture Stimulator JM-3A by Dr. Xie Huisheng

3. Results

3.1. Treatment and results

Unfortunately, OA is a progressive disease and there is no known cure for it*[3]*. The best way to keep a dog's joints healthy is to prevent the reproduction of the ones already diagnosed with hip dysplasia, weight management and prevention of obesity and joint supplements in order to reduce inflammation and slow progression of joint damage*[3]*. NSAIDs remain a popular treatment for OA despite their well-known side effects that may occur with their long-term use*[1]*.

In the case of Rufus, he was on Mavacoxib also known as Trocoxil, for more than two years, one dose every month. He was also on chondroprotective such as Glucosamine and Chondroitin and gastric protectors. From time to time he developed diarheea and capricious appetite, which we assume might be side effects of NSAIDs.

Since the condition appeared to be amenable to Ac therapy and had not previously responded well to conservative treatment, the owner decided to pursue Ac treatment (Figure 2 and 3). Alternatively, conservative management could be continued with chondroprotective.

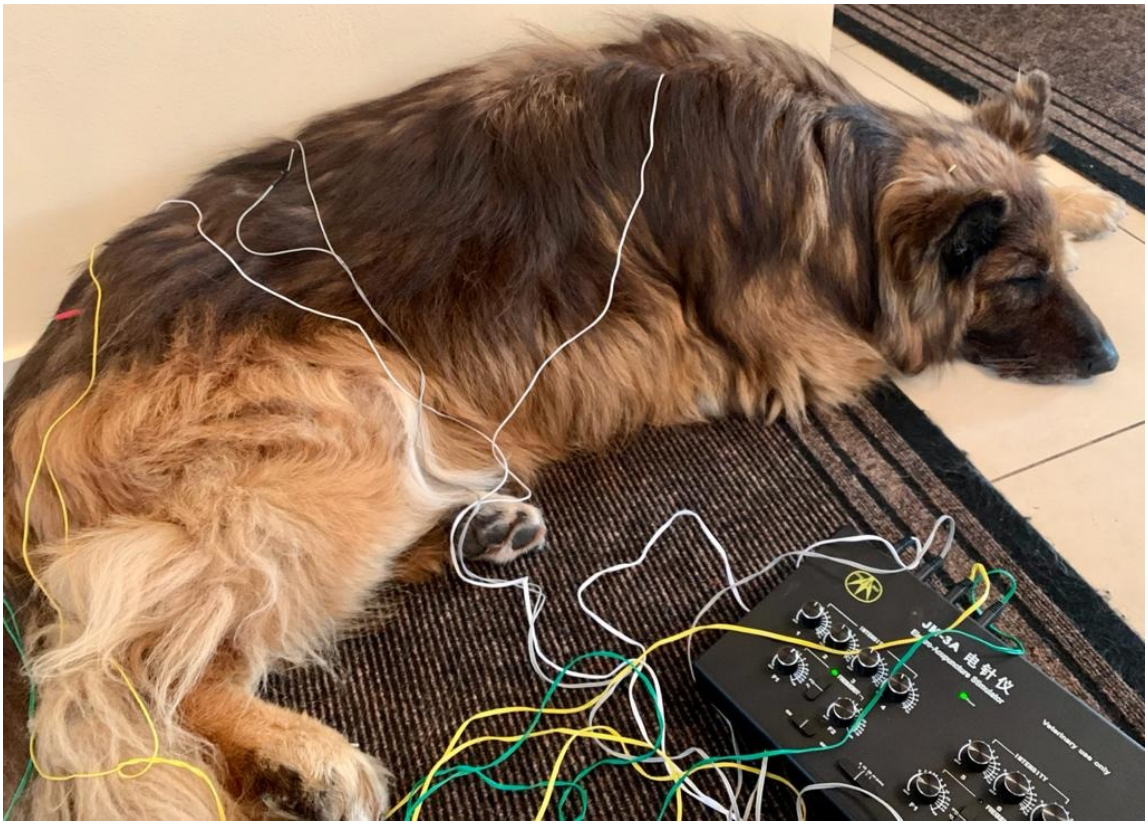

Figure 2. Rufus sleeping during the electroacupuncture treatment.

Figure 2. Rufus during the treatment: LIV3 (bilaterally), KID3.

During the winter, weekly treatment was planned in order to reduce the inflammation and also the side effects that may occur once the cold weather is coming. Most of the time, the dog stays outside the house. During each treatment, the dog relaxes and starts to sleep. The owners said that he had slept several hours after each session.

After the fourth treatment, the dog started to have more energy, enjoys long walks and playing. Also, now he enjoys being rubbed on his back. Most important, we've managed to stop the administration of NSAIDs for 6 months since we started the acupuncture treatment until the present. The owners are very pleased with the evolution of their dog. We've managed to improve his life quality.

The Kidney Meridian represent the root of prenatal life and holds the Jing, also known as life essence. Moreover, Kidney along with Bladder control water metabolism and regulates its excretion, and dominates the bones and marrow. As the animal increases with age, the Qi is consumed and the Kidney Patterns include diseases such as urinary incontinence, intervertebral disc disease or arthritis *[4]*. Each acupoint has a specific role in the treatment of this pathology. The aim of each acupuncture treatment is to re-establish the equilibrium of the whole body. Some of the most used acupoints in treating OA are represented in the adapted Figure 3 *[6]*. We must remember that this type of treatment should be used only by trained practitioners in order to have the best results.

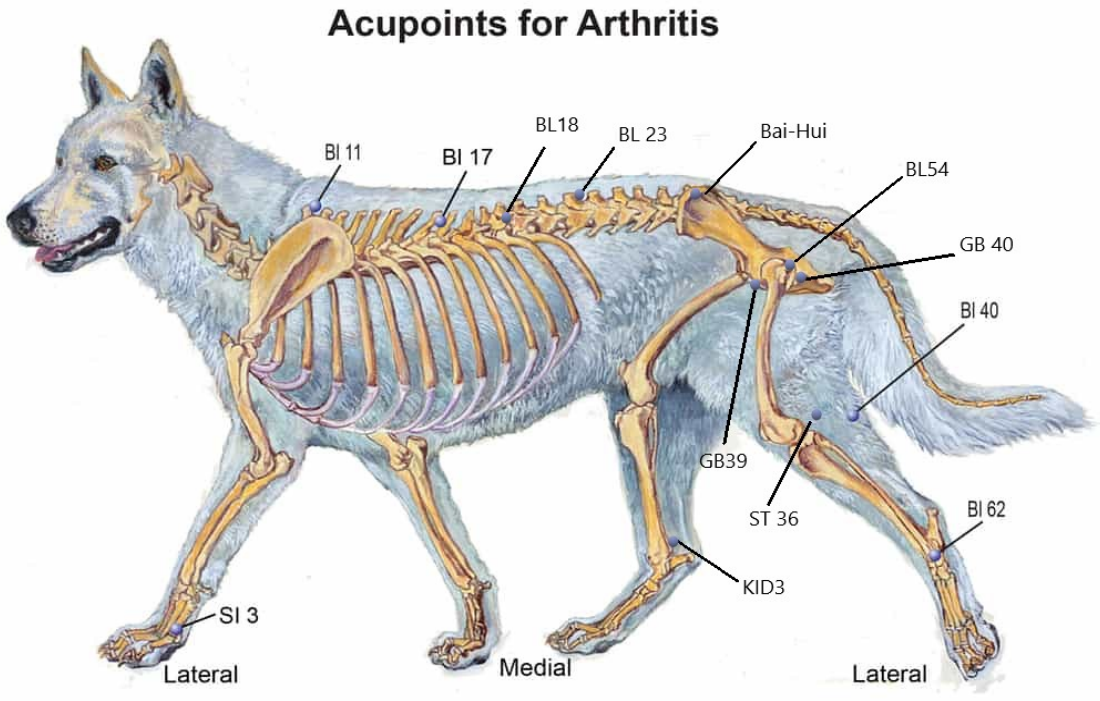

Figure 3. Some of the acupoints used for treatment. Adapted after *[6]*.

4. Discussion

The aim of this study is to bring relevant information and to describe a complementary method for the treatment of OA in dogs.

Western medicine is described as a medical method to treat a disease process, while TCVM is more about creating a balance with Qi flow through the body**[5]**. Each of them has strengths and weaknesses,

but the main purpose of both is to cure diseases, this is one of the reasons why TCVM is considered complementary medicine. As so, TCVM continues to change and to adapt as the new medical information are developing into the medical field**[4]**.

From a Western point of view, an alternative could have been surgery, which would involve total hip replacement to treat his hip dysplasia. However, because he would need both hips to be replaced, the recovery time and the costs would have been too much. The owners would not afford it, especially because of the risk involving the anaesthesia.

Another treatment option could include administration of NSAIDs such as Meloxicam, Mavacoxib, Carprofen or Robenacoxib, but in time, this type of drug has side effects, especially in the gastrointestinal tract**[1,7]**. According to Monteiro-Steagall's**[8]** study about the side effects of NSAIDs, more than 50% of the studies reported adverse effects. Of course, we cannot exclude these treatments in the acute phase of OA. On the other hand, Teixeira et al.'s study showed neither acupuncture nor carprofen differs significantly. Both treatments reduced the degree of lameness, while acupuncture was associated with a decrease in validated chronic pain scores**[9]**.

From a TCVM point of view, Rufus was diagnosed with Bony Bi Syndrom, or Kidney Yang and Qi Deficiency. A Kidney Qi Deficiency Pattern is, generally described as an animal's condition in old age, weakness with difficulty in rising. The Kidney dominates the lower back and hind limbs, and when there is a problem with Qi flow in this area, a deficiency might occur. General speaking, a chronic condition with Kidney Qi Deficiency leads to Kidney Yang Deficiency in time, if not treated[4]. In other words, we will have an animal with difficulty in standing up or lying down, cold back, hind limbs and ears and warm seeking (the animal want to stay in warm places, lay in the sun, or even refuse to go outside if is rawish)[10].

EA is a superior method of stimulating specific acupoints by using electric current applied through the needles. EA can be applied with different machines that have the ability to offer a wide range of amplitude and frequency[5]. Furthermore, you can customize each treatment. When compared to Ac, EA has the advantage of shorting the treatment time and delivers a better level of stimulation[5].

AP was made with Zeel, once a month. Zeel offer a safe and effective homeopathic alternative. It is the treatment for hip osteoarthritis, joint pain, bone fracture and stiffness from mild to moderate and high form. Zeel is particularly effective in relieving the symptoms associated with degenerative arthritis[14]. It is made of plants and was used as an injection in certain acupoints.

The mechanism involved in acupuncture includes pain management through muscle relaxation, reducing compression in the joints, but also increasing blood flow with oxygen to the tissues. All these actions translate into reducing inflammation at the site of acupuncture[9,11].

Mechanisms of acupuncture involve increases in plasma β-endorphin concentration, which is responsible for inhibiting presynaptic pain transmission. Also, serotonin is involved in relieving pain[12]. In addition to the anti-inflammatory and immune-boosting effects, acupuncture alleviates physical and emotional stress[13]. Furthermore, it accelerates the process of healing tissues through the release of endorphins and serotonin[12,13].

The limitations of this study are related to the need for more accurate parameters regarding the effect of acupuncture. In the future, studies are needed on larger samples of dogs with OA, respectively, more comparative studies between acupuncture, electroacupuncture and Western medicine.

5. Conclusions

EA confers excellent analgesia and lessens the time needed to regain normal neurologic function, but what we must not forget is that rigorous training of the acupuncturist is required to have good results. Each acupoints has a precise position, and the treatment plan is chosen according to several parameters within which TCVM works.

6. Patents

Author Contributions: Conceptualization, M.F.D; Writing- original drag preparation, M.F.D., Supervision, C.P.P. and L.O. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Acupuncture points used and their clinical relevance[4]**:**

- GV20: Calming point, Shen Disturbance;
- GB29/GB30: Gluteal muscle soreness, pelvic limb pain, arthritis of coxofemoral joint;
- KID3: Dyspnea, deagness, back pain;
- KID6: Insomnia, dysuria, Yin Deficiency;
- BL54: Master point for pelvic limbs, hip problems, lumbar pain;
- BL23: Association point for Kidney Qi Deficiency, deafness, back pain;
- BL20: Association point for Spleen, abdominal fullness, back pain;
- BL18: Association point for Liver, back pain, epilepsy;
- BL11: Influential point for Bone, cervical stiffness, intervertebral disc disease, back pain;
- ST36: Master point for gastrointestinal tract and abdomen, gastric pain, general tonic;
- Bai-Hui: Yang Deficiency, intervebral disc disease, pelvic limb paresis;
- Shen-Shu/Shen-Peng/Shen-Jiao: Yang Deficiency, pelvic limb paresis or paralysis.

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CVJ CLUJ VETERINARY JOURNAL

Communication

Brain auditory evoked response test, the standard method for the diagnosis of the hereditary deafness in dogs

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Abstract: Hearing deficiency is one of the most common hearing impairments that affect humans and other mammalian alike. Hearing loss is not painful or a life-threatening change but can endanger the patient by taking into account a large number of breeds predisposed to hereditary deafness, this short communication aims to synthesize the steps and the method for BAER test. For the affected breeds, the BAER test is recommended starting at the age of two months.

Keywords: BAER, dog, auditory evoked response test

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1. Introduction

Dog breeds that are affected by hereditary deafness: Akita, Dalmatian, Nova Scotia, Duck Tolling Retriever, Alapaha Blue Blood Bulldog/Otto Bulldog, Dappled Dachshund, Old English Sheepdog, American Bulldog , Doberman Pinscher, Papillon, American-Canadian Shepherd, Dogo Argentino, Pekingese, American Eskimo, English Bulldog, Perro de Carea Leones, American Hairless Terrier, English Cocker Spaniel, Pit Bull Terrier, American Staffordshire Terrier, English Setter Pointer/English Pointer, Anatolian Shepherd, Foxhound, Presa Canario, Australian Cattle Dog, Fox Terrier, Puli, Australian Kelpie, French Bulldog, Rhodesian Ridgeback, Australian Shepherd, German Shepherd, Rat Terrier, Australian Stumpy-tail Cattle Dog, German Shorthaired Pointer, Rottweiler, Beagle, Goldendoodle, Saint Bernard, Belgian Sheepdog/Groenendael, Great Dane, Saluki, Belgian Tervuren, Great Pyrenees, Samoyed, Bichon Frise, Greater Swiss Mountain Dog, Schnauzer Border Collie Greyhound Scottish Terrier Borzoi Havanese Sealyham Terrier Boston Terrier, Ibizan Hound, Shetland Sheepdog, Boxer, Icelandic Sheepdog, Shih Tzu, Brittney Spaniel, Italian Greyhound, Shropshire Terrier, Bulldog Jack/Parson Russell Terrier, Siberian Husky, Bullmastiff, Japanese Chin, Soft Coated Wheaten Terrier, Bull Terrier, Keeshond, Springer Spaniel, Canaan Dog, Kuvasz, Sussex Spaniel, Cardigan Welsh Corgi, Labrador Retriever, Tibetan Spaniel, Catahoula Leopard Dog, Lhasa Apso, Tibetan TerrierCatalan Shepherd LowchenToy Fox Terrier Cavalier King Charles Spaniel Maltese, Toy Poodle, Chihuahua, Manchester Terrier, Walker American Foxhound, Chinese Crested Miniature Pinscher, West Highland White Terrier, Chow Chow, Miniature Poodle, Whippet, Cocker Spanielmongrel, Yorkshire Terrier, Collie, Newfoundland Landseer, Coton de Tulear, Norwegian Dunkerhound [1].

For these dogs, the BAER test is recommended starting at the age of two months.

This paper aims to describe the technique of the test and the interpretation of the results. We will not insist on the etiology of deafness as it is described elsewhere [2,1,3]. However, some elements of classification, anatomy, physiology, and genetics are necessary.

2. Deafness

Deafness can be peripheral or central, congenital / inherited or acquired, uni- or bilateral, total or partial, conductive or neurosenzorial.

The congenital deafness should not be confused with the hereditary one; the congenital form may appear during parturition and is determined by dystocia and oxygen deprivation. Hereditary deafness occurs 3 to 4 weeks after birth and is a neurosensorial type. It is the result of the degeneration of a structure called stria vascularis. This structure is responsible for maintaining a very specific composition of the endolymph from scala media, characterized by a high concentration of potassium ions and a low concentration of sodium ions, different from other extracellular fluids inside the body. This process is involved the numerous melanocytes present in stria vascularis[3,4].

When the atrophy of the stria occurs and/or the melanocytes are missing, the composition of the endolymph is altered and leads to the death of the hair cells from the organ of Corti. The hair cells are the ones who transformed the sound (mechanic oscillations) transmitted to the cilia by the tectorial membrane in electric impulses. When these structures are damaged, peripheral neurosenzorial deafness appears.

A different situation is described in Dobermans when the hair cells die, but the melanocytes from stria vascularis are intact, called neuroepithelial deafness [5,6].

The inheritance of deafness is controlled by a gene situated on Merle (M) locus, with two alleles: the recessive m and the dominant M. The homozygous MM individuals present blue irises and may be blind and sterile. Unfortunately, heterozygous Mm x Mm parents will give birth to 25% MM puppies.

Another gene is placed on the S locus, and it is called piebald. It has four alleles: dominant (S) and recessive (s^i) Irish spotting, (s^p) piebald, and (SW) extreme white piebald. The inheritance mechanism is considered to best fit by the presence of two autosomal recessive genes or an incompletely penetrant recessive allele.

Regarding the deafness in Dobermans, the inheritance has an autosomal recessive mechanism [3].

3. BAER test

According to the "Orthopedic Foundation for Animals," the only accepted test (at least for the moment) to detect hereditary deafness in dogs is the BAER (Brain Auditory Evoked Response) test.

Headphones deliver the test sound and one ear at a time test can be diagnostic for other types of acquired deafness (toxic, traumatic, degenerative, inflammatory, etc.) (fig. 1). It is recommended to perform the test at the age of two months. The test shows a few waves, and the first five ones are of diagnostic importance. The waves are marked with roman numbers (fig. 1).

Wave I is generated by the distal end of the cranial nerve VIII, wave II by the proximal part of cranial nerve VIII, wave III by the cochlear nucleus, wave IV and V inferior colliculus, and probably by the medial geniculate body. Anyway, the disturbances responsible for altering the last two waves will determine other symptoms of diseases of the central nervous system.

The test can be done without sedation, but if the patient is restless, it can be sedated because the medication does not affect the test result. Currently, we are using four electrodes: one "grounding" electrode at the level of the T1 vertebra, one positive electrode on the median line of the skull at the level of the ears, and two negative electrodes anterior to the tragus of each ear.

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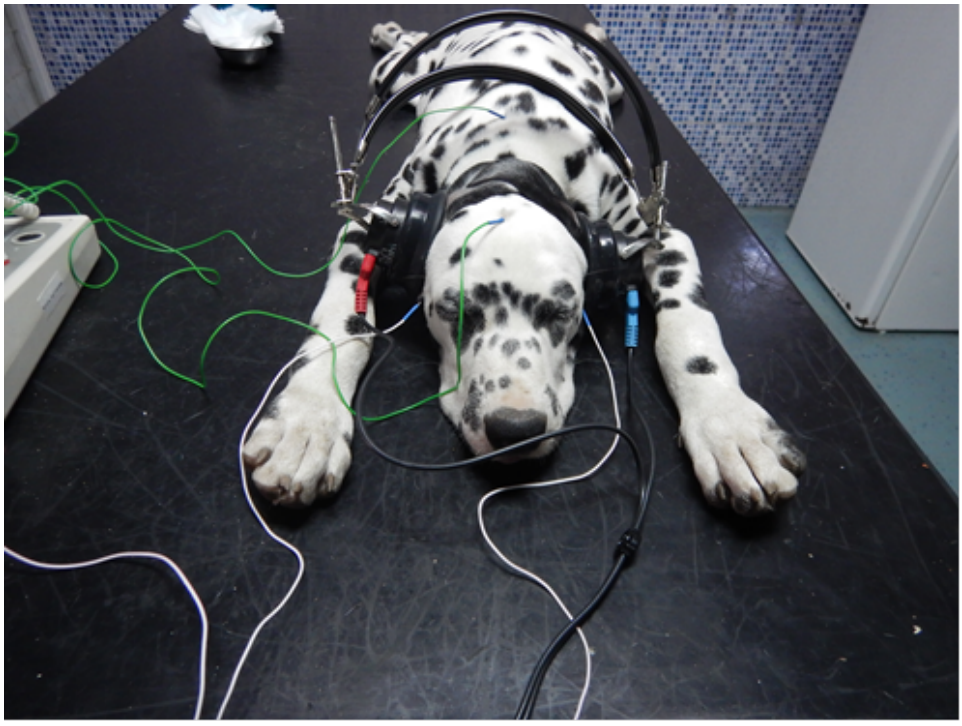
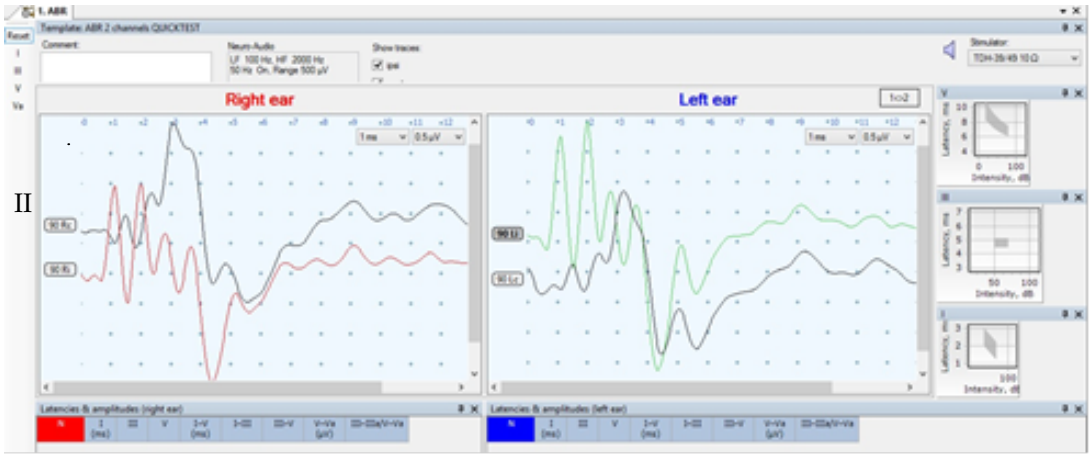


Fig. 1 Positioning of headphones in BAER test

I



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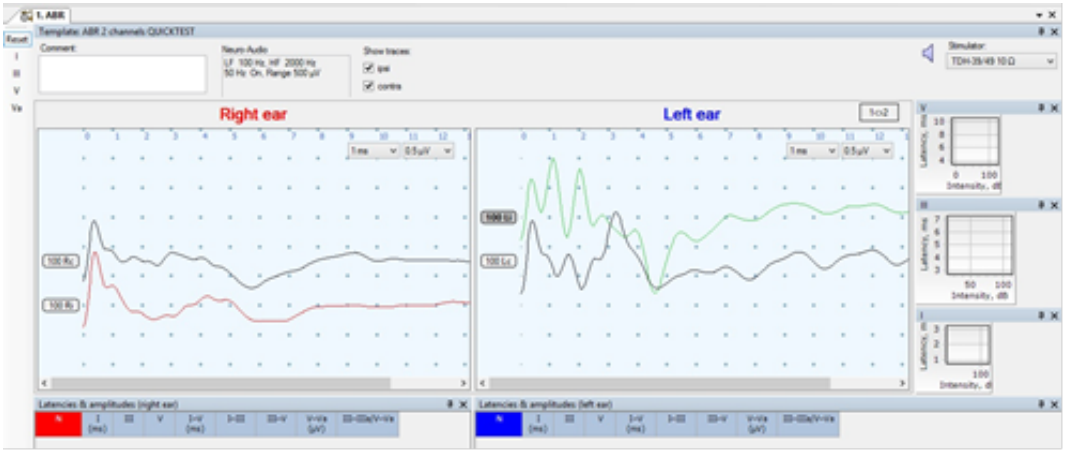

Fig. 2 Right ear deafness.

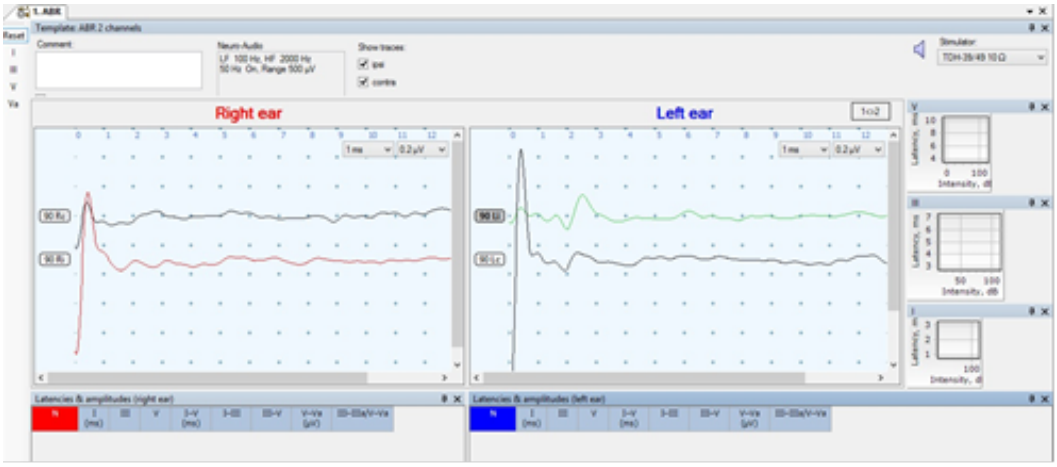

Fig. 3 Bilateral deafness.

To avoid motion artifacts, sedation of the patient is recommended.

Fig. 4 Motion artifact

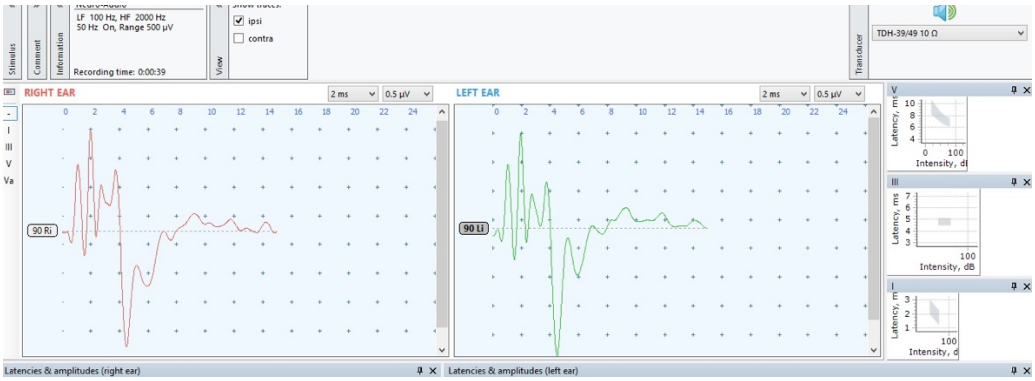

Fig. 5. Same patient after sedation.

4. Discussion

Identification of the dogs (e.g., microchip) is very important because uni- or bilateral deaf dogs must be excluded from breeding. Puppies with unilateral deafness can be excellent pets, but those with bilateral deafness need special attention. These dogs can be good companion animals, but care should be taken to avoid unattended contact with other persons or animals. Because they cannot hear, they may have a violent reaction when touched without warning. Anytime someone wants to pet the dog, he should be sure that he is within the visual range of the affected dog. It is recommended to provide these patients with collars or harnesses with warnings (e.g., Don't touch! Deaf dog!) One can find/buy this type of equipment in pet shops or on the internet. It is highly recommended to use them, to avoid accidents.

Conflicts of Interest: None

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