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The ICU patient: from reanimation to pain therapy – personal experiences

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Abstract

This thesis wants to be a contribution to the current knowledge in the Emergency and Critical Care medicine, by focusing on different aspects varying from the reanimation to the pain therapy of ICU patients. The thesis has been organized in different Sections, regarding the cardiovascular and respiratory systems, and haematology, respectively.

The first Section, which focuses on the cardiovascular system, consists of two different clinical studies. The first describes the effect of a Limited Fluid Volume Resuscitation (LFVR) protocol in ten cats with non cardiogenic shock, unresponsive to the initial Conventional Resuscitation (CR) with isotonic crystalloids. Cats were fluid resuscitated with up to 8 mL/kg of hypertonic saline (HS: 7% NaCl) and up to 8 mL/kg of hydroxyethyl starch (HES). Each bolus was administered over 5-10 minutes and patients vital signs were re-evaluated, for additional boluses, every 5-10 minutes until stabilization. Animals were considered stable once the vital parameters (heart and respiratory rates, temperature, sensorium and quality of the pulse) were within the physiological ranges. The stabilization of the vital parameters occurred in 33±12.7 minutes (15-60 minutes) in all cats. Of the ten cats, six currently enjoy good health (6-25 month follow up).
The aim of the second study was to assess epidemiology and echocardiographic findings of pericardial effusion (PE) in canine patients and to determine the clinical usefulness and safety of a new pericardiocentesis technique, using a "fistula needle" for haemodialysis. On a database of 5304 dogs, PE was identified in 91 dogs (1.71%). The most common causes of PE were neoplasm in 33 cases (36.26%) and a severe degenerative mitral and tricuspid degenerative valve disease in 32 cases (35.16%). Echo-guided pericardiocentesis, using a "fistula needle" for haemodialysis, was performed in 28 cases (30.77%) with cardiac tamponade. No adverse effects were found in any of the patients during the following 48 hours of follow up.

The second Section, regarding the respiratory system, consists of two different case reports. The first one was about a case of severe shock and haemothorax, due to anticoagulant rodenticides poisoning, treated with autotransfusion. The second report was about a case of acute hypoxaemic respiratory failure with haemoptysis in a dog exposed to copper sulphate powder.

The third Section describes one case report of a dog with aplastic pancytopenia associated with multiple CVBDs, successfully treated with cyclosporine and aetiological treatments, and a case series of four dogs with Canine Adenovirus (CAV-1) infection, alone or together with Canine Parvovirus (CPV)

The last Section was destined to the topic of pain assessment and analgesia. The first chapter of the Section describes the findings of a survey on Italian veterinarians knowledge and attitude towards pain assessment and analgesia, with the aim of assessing if any regional variation exists between the South and the North of Italy and if analgesia is an adequately considered topic in the South of Italy. The results of the survey and of the statistical analysis, showed no significant differences between the South and North groups, except for the greater use of meloxicam, methylprednisolone and local anaesthetics in the South group, whereas buprenorphine, butorphanol and ketamine were more used in the North group.

The last chapter describes two clinical studies carried out on dogs undergoing hemilaminectomy for acute thoracolumbar intervertebral disc extrusion and on dogs with Degenerative Lumbosacral Stenosis (DLSS), respectively. In both study, the Short Form of the Glasgow Composite Pain Scale (GCPS-SF) was applied to evaluate pain score. Based on the results of the first study, which compared the analgesic activity of buprenorphine and tramadol, both drugs showed a good analgesic activity. However, buprenorphine showed a faster and greater analgesic effect, compared to tramadol. None of the two molecules showed any side effect. In the second study, the analgesic conservative treatment of DLSS was started with tramadol. However, after the first week of treatment, the dogs of Group A were considered to be non-responder to tramadol. For this reason, the analgesic treatment was switched to gabapentin. After that, no statistical differences have been found between the two groups. Already after the 2nd week of treatment, GCPS scores were considerably lower in both groups, suggesting that both tramadol and gabapentin could be effective in reducing lumbosacral pain in dogs with DLSS. Moreover, the improvement of muscle tone in the group treated with gabapentin after 4 weeks of follow-up could be due to the resumption of a moderate physical activity determined by a better pain control than in dogs treated with tramadol.

Veterinary patients may be admitted to an Intensive Care Unit (ICU) because of different pathological conditions, that may represent a life-threatening hazard for the patient if not promptly and properly managed, as described in the cases reported in this thesis. For these reasons, the ICU doctor should have knowledge on different medical fields and should be able to take prompt decisions on the diagnostic tests and treatments to perform. “Time is money” but, in a critical patient, can also make the difference between life and death.

PREFACE

Emergency and critical care has been a growing field in small animal practice over the last 20 years. Emergency clinicians have to face with several life-threatening conditions and must be able to take prompt decisions regarding clinical, diagnostic and surgical interventions, with the main goal of saving patient's life. The appropriateness of these decisions can make the difference between life and death. This approach represents the main diversity of Emergency which focuses on monitoring patient's vital parameters, preventing the onset of possible complications and keeping the patient alive. Intensive Care medicine, instead, has the aim of investigating the underlying clinical problem of every patient, in order to reach a definitive diagnosis.

The definition of emergency patient refers to a patient who suffers from an acute or chronic condition which make the clinical status of the animal unstable and may pose an immediate risk for his/her life. This means that emergencies may vary from minor issues to patients that are close to death. Moreover, ICU patients are often sick since a long time and are referred from smaller practices, where the animal has already been treated.

Although nowadays, more and more specialist Emergency and Intensive Care Units (ICU) exists, most of the emergencies are primarily seen by first line veterinary practitioners. For this reason, it is very important that every veterinarian should have, at least, the basic knowledge on how to deal with an emergency patient. However, although smaller practices should be able to provide urgent stabilization, they should also be able to recognize when complicated conditions need to be referred to a more specialised ICU.

All the staff of an Emergency Service has to be well prepared on how to approach an emergency patient. Preparation includes taking prompt and resolute decisions, but also having the right equipment close to hand and ready to use. All the members of the emergency team must be aware of the specific protocols and know exactly what is their role and the tasks they

are delegated to. The environment should be appropriated, with a well-equipped central location to triage and stabilise the patient. Having a good communication both within the team and with the owner, is also a primary important factor. Even during the following hospitalization, sudden changes in the clinical status of critical patients can occur at any time, and the period over which it is possible to act in order to reduce morbidity and mortality, may be really short. For this reason, every loss of time due to the lack of organization or to the search of the needed instruments, can result in a negative outcome.

Regarding the skills that all the members of emergency and ICU teams should have, *triage* and stabilization of the major body systems are on the top of the list. The word *triage* refer to the process of rapidly classifying patients on the basis of the severity of their clinical condition, allowing patients with life-threatening conditions to be seen before patients with less severe problems. *Triage* should be performed for every patient, within 5-10 minutes from the admission and involves the collection of the first information from patient's history and the evaluation of the cardiovascular, respiratory and neurological body systems. In fact, severe life-threatening conditions involving these major body systems can suddenly deteriorate and cause the patient's death.

Immediately after the triage examination, urgent empirical stabilisation (e.g. oxygen administration and fluid therapy) has to be started according to the patient's need. In the meantime, further diagnostic has to be carried out in order to identify the life-threatening problem and to formulate a specific treatment plan.

Among the conditions that require an emergency examination, severe pain is one of the most common. Moreover, ICU patients often show various degrees of pain, which can determine several detrimental effects on different body systems that may have a severe impact on the outcome. Although analgesia may be postponed for a severely injured patient due to the need for immediate lifesaving interventions, adequate pain control is ultimately essential to offset further detrimental effects. Moreover, pets are now seen by the owners as members of their

family. Most of the owners take into account and appreciate the way the veterinary practitioner approaches their animal. By being kind, sensible and respectful of the animal's clinical condition, the patient may be better helped to deal with a pathologic disease and a new environment that could result in a very stressful situation, and the owner could be more satisfied, despite the final outcome. In this perspective, pain management has also the aim of avoiding or, at least, reducing, animal suffering, and of ensuring animal welfare.

This thesis wants to be a contribution to the current knowledge in the Emergency and Critical Care medicine, by focusing on different aspects varying from the fluid resuscitation to the pain therapy of the ICU patient.

The thesis has been organized in different Sections, regarding the cardiovascular and respiratory systems, and haematology, respectively. The last Section was destined to the topic of pain assessment and analgesia. Every Section has been divided into different chapters, each one reporting different studies carried out during the PhD course, regarding resuscitation fluid therapy, management of pleural effusion, and pain management in dogs with neuropathic pain. The last Section includes a survey on the attitude and knowledge of Italian veterinary practitioners toward pain assessment and analgesia. Finally, case reports or case series describing uncommon emergency conditions that have occurred in the abovementioned period, have been included in the relative sections.

I. CARDIOVASCULAR SYSTEM

1.1 Limited fluid volume resuscitation (LFVR) in severe shock unresponsive to initial fluid challenge: a preliminary study in ten cats

Shock is a complex syndrome caused by a limited organ blood perfusion with a consequent reduction in the availability of oxygen tissue level that, if not quickly handled, can determine patient's death. It may be a result of many diseases and it is a common condition in critically ill patients. Intravenous fluid resuscitation plays a primary role in the treatment of circulatory failure in the emergency and intensive care unit settings (Cazzolli and Prittie, 2015). The goals of fluid treatment include the restoration of plasma volume and organ perfusion, and the rapid control of the main underlying problem. The so-called "triad of shock" (bradycardia, hypotension and hypothermia) is a common condition in cats with hypovolemic shock. It is assumed that in cats, unlike other species, bradycardia development instead of tachycardia in response to a state of hypotension is due to the simultaneous activation of vagal and sympathetic fibres from the aortic and carotid baroreceptors (Hackett, 2015; Schwartz et al., 1973). Moreover, the adrenergic receptors become refractory to catecholamines at a body temperature below than 37.5 °C, perpetuating both bradycardia and secondary compensatory vasoconstriction (Brady et al., 2000).

Intravenously administration of every type of fluid determines an immediate plasma volume expansion. However, each type of fluid owns different systemic effects that could affect patient outcome (Myburgh and Mythen, 2013). Every type of fluid has its own safety and toxicity profile and their pharmacologic properties can influence organ function, alter coagulation, modulate the immune system and affect blood viscosity, red blood cell rheology and endothelial function (Cazzolli and Prittie, 2015).

Conventional resuscitation (CR) consists in the administration of large volumes of isotonic crystalloid solutions (60-90 mL/kg/h in dogs and 40-60 mL/kg/h in cats), although it is currently recommended to start with 1/4 or 1/3 of the total volume and re-evaluate the patient before administering additional fluids (Driessen and Brainard, 2006; Rozanski and Rondeau, 2002). Another protocol used in clinical practice, is to administer 15-20 mL/kg of isotonic crystalloids in rapid bolus in dog and within 15 minutes in cat (for the higher risk of pulmonary oedema caused by fluid overload), until a maximum of four boluses in dogs and three in cats, re-evaluating the patient every fifteen minutes (Viganò, 2011). Disadvantages of CR consist in: i) prolonged administration time; ii) rapid redistribution of isotonic crystalloids into the interstitial space (60-80% of the administered fluid within 30-60 minutes), with the risk of oedema formation; iii) hypothermia and iiii) risk of aggravation of any bleeding caused by the destabilization of clots and dilution of the circulating clotting factors (Driessen and Brainard, 2006). The positive fluid balance that could follow aggressive resuscitation with crystalloids is associated with worsened patient outcome. The expansion of extracellular spaces determines a damage of the endothelial surface layer so causing capillary leak and tissue oedema (Cotton et al., 2006). Moreover, isotonic crystalloids could induce a pro-inflammatory state, with an increase in cytokine production and endothelial cell activation, worsening oedema formation (Cazzolli and Prittie, 2015).

Colloidal solutions have a volume-sparing effect with a decreased risk of inducing a positive fluid balance. This type of fluid contains large molecules that cannot cross an intact vascular barrier, so decreasing the possibility of tissue oedema formation, and has a longer plasma half-life. However, adverse effects such as coagulation disorders, acute kidney injury (AKI), pruritus, hepatopathies and anaphylactoid reactions have been reported as adverse effects which could follow the administration of colloidal solutions (Cazzolli and Prittie, 2015).

These problems led to the research of alternative treatments such as the Limited Fluid Volume Resuscitation (LFVR), also known as low-volume or small-volume fluid resuscitation

(Hammond et al., 2014; Hammond and Holm, 2009; Rocha-e-Silva and Poli de Figueiredo. 2005; Stern, 2001; Muir and Sally, 1989). The LFVR protocols, involving the use of a hypertonic saline solution and/or colloids, are based on the use of a smaller volume of fluid able to restore blood volume and to solve the shock, thus minimizing the risk of interstitial fluid extravasation and clots breaking (Hammond and Holm, 2009). The level of blood pressure reached in LFVR is lower than in CR. A mean arterial blood pressure of 70 mmHg or a systolic arterial blood pressure of 90 mmHg is still enough to keep perfusion of vital organs, with reduced risk of bleeding (Hammond and Holm, 2009). Several studies on hypovolemic shock, in animal models and humans, evaluated different protocols of resuscitation fluid therapy and highlighted how the combination of hypertonic saline and colloids produces best effects on the increase of mean arterial blood pressure, oxygen saturation and cardiac output compared to isotonic crystalloids (Wang et al., 2015; Hammond et al., 2014; Watters et al., 2006; Friedman et al., 2003; Mapstone et al., 2003; Schertel et al., 1997; Muir and Sally, 1989). However, only a few studies have been carried out on the use of HS solution and LVFR protocol in cats (Dupe et al., 1993; Muir and Sally, 1989).

1.1.1 Aim of the study

The aim of this study was to evaluate the effect of a LFVR protocol, with HS alone or combined with HES administration, in cats with severe not cardiogenic shock, unresponsive to initial CR with isotonic crystalloids.

1.1.2 Materials and method

Protocols of animal husbandry and experimentation were reviewed in accordance with the standards recommended by the *Guide for the Care and Use of Laboratory Animals* and Directive 2010/63/EU for animal experiments and were approved by the “Ethics Committee”

of the Veterinary Department of the University of Messina (record number 009/2016). This study was carried out in accordance with the Good Clinical Practice.

Case selection criteria

The study was carried out in the Veterinary Teaching Hospital of the University of Messina, in the period between November 2014 and November 2016, on cats referred in the emergency service with a red triage code for shock of various origins unresponsive to initial CR. Cats were enrolled in the study after the informed consent has been provided by the owners.

Animals

During the study period, 60 cats in shock have been referred to the emergency service. The selection criteria included the presence of a non-cardiogenic shock due to various causes and the unresponsiveness to the initial CR protocol with isotonic crystalloids. Exclusion criteria were the presence of cardiac signs (murmur, gallop rhythm, muffled heart sounds, distended jugular and jugular pulse) and dyspnoea on physical examination, and the administration of glucocorticoids, vasopressors and mannitol prior to or on admission.

Procedure

Shock was established on the basis of alterations of three or more of the following objective parameters: heart rate (HR) <140 or >225 beats/minute (bpm); capillary refill time (CRT) >2 seconds; respiratory rate (RR) >40 breaths/min; rectal temperature (T°) <37.8 °C; blood lactate > 2.5 mmol/L (laboratory range: 0.5-2.5 mmol/L). Subjective parameters (*Kirby' Rule of 20*), like pulse quality, mucous membranes colour, state of the sensorium and temperature of the extremities (Mazzaferro, 2013) were recorded, too. When it was possible, a micro venous withdrawal was performed, in order to determine the following parameters: packed cell volume (PCV), by means of capillary tubes for microhaematocrit; plasma total solids (TS), by means of a refractometer; lactate, by means of a portable blood lactate meter (Lactate Scout +, EKF Diagnostics, Penarth, Cardiff); glucose, by means of a portable blood glucose

meter (Glucocard, Menarini, Firenze, Italy). Immediately after the presentation, a continuous supply of oxygen flow-by using an oxygen concentrator was provided to all the animals. When needed, patients were re-warmed and the rectal temperature was recorded continuously using a probe connected to a monitor.

Fluid resuscitation protocol

A venous (cephalic vein) access was achieved and 15-20 mL/kg of lactate Ringer's solution (LRS; Ringer lactate solution S.A.L.F. S.p.A., Cenate Sotto, Bergamo, Italy), preheated (38 °C), were administered over 15 minutes. When a vascular access was not immediately available, an intraosseous (femoral trochanteric fossae) catheterization was performed.

Cats that did not show any improvement of the vital parameters within 10 minutes after the end of the initial CR with isotonic crystalloid, were selected for the administration of the LVFR protocol.

Hypertonic saline solution (HS) was prepared from a commercial solution of 11.7% NaCl (10 mL vials, NaCl 2 mEq/mL, Galenica Senese, Monteroni D'Arbia, Siena, Italy). The dilution was prepared by adding, under sterile conditions, 3 mL of the concentrated solution to 2 mL of sterile water for injections, obtaining a final concentration of 7.02%. Animals could receive up to a maximum of 8 mL/kg of HS and 8 mL/kg of hydroxyethyl starch (HES; Voluven®, Fresenius Kabi Italy S.r.l., Isola della Scala ,Verona, Italy). Boluses of 2 mL/kg were administered over 5-10 minutes and patients vital parameters were re-evaluated every 5-10 minutes until stabilization and, then, every 30 minutes. Additional boluses were administered 10 minutes after the end of the previous bolus, if the vital parameters were still abnormal. Cats were resuscitated until the achievement of the desired levels of vital parameters and not until the infusion of a fixed volume of fluid. The main purpose of assessment was the time (minutes) until the stabilization of vital parameters.

Animals were considered stable once the vital parameters (temperature, heart rate, respiratory frequency, quality of the pulse and sensorium) were within the physiological ranges of the

species. In particular, animals were deemed stable after achieving the following stabilization endpoints: T >37.8 °C; HR >140 and <225 bpm; CRT <2 sec; RR <40 breaths/minute; full and strong pulse; alert and bright sensorium; warm extremities. When possible, serial micro-withdrawals for the PCV, TS, lactate and glucose levels were performed every 30 minutes until stabilization, and 30 minutes later.

After stabilization, animals were hospitalized and underwent any other additional investigation (blood chemistry, haematology, ultrasound and radiological diagnostic evaluations) in order to establish the underlying problem. Additional supportive care and treatments were at veterinary practitioner discretion.

Statistical analysis

The time until stabilization (minutes), the volume of HS and HES administered and the effect of LFVR on heart rate (HR), rectal temperature (T) and respiratory frequency (RR) were determined. A descriptive statistical analysis was applied for age, body weight, volume of HS and HES administered and time until stabilization of the ten cats enrolled in the study. One-way analysis of variance (ANOVA) for repeated measures was applied to evaluate the effect of LFVR protocols on vital parameters (HR, T and RR) throughout the experimental period. The parameters recorded before (T0), every 10 minutes during the first 30 minutes (T10, T20, T30) and 60 minutes (T60) after LFVR protocol was started, were used for the statistical analysis. When significant differences were found Bonferroni's post hoc comparison was applied. Statistical significance was set at p values <0.05.

Data were analysed using the software STATISTICA 7 (Stat Soft Inc., USA, 2003).

1.1.3 Results

During the study period, ten client-owned cats met the selection criteria. Among them, nine were Domestic Shorthair and one was Domestic Longhair, six were male (one neutered) and

four female (two neutered), aged between 1 month and 10 years (mean±SD: 27.9±41.7 months; median: 5.5 months) and body weight from 0.26 to 5 kg (mean±SD: 2.3±1.9 kg; median: 2.2) (Table 1).

Table 1. Breed, gender, age and body weight of the cats enrolled in the study

Case n°	Breed	Gender	Age	Body weight (kg)
1	DSH*	NF‡	8 months	4.1
2	DLH†	M	1 month	0.55
3	DSH	NF	7 years	3.2
4	DSH	M	2 years	4.5
5	DSH	M	3 years	5
6	DSH	M	1 month	0.26
7	DSH	M	1 month	0.32
8	DSH	F	1 month	0.74
9	DSH	M	10 years	3.2
10	DSH	F	3 months	1.1

*DSH, Domestic Short Hair: †DLH, Domestic Long Hair: ‡NF, neutered female

On admission, all the cats were bradycardic (HR 96.6±21.9 bpm), with a weak pulse, pale to white mucous membranes, CRT ≥3 seconds and depressed to stuporous sensorium; nine were hypothermic (T: 32.8±3.3 °C) and seven tachypnoeic (RR 50.4±22.6 breaths/minute) (Table 2). Conventional fluid resuscitation was initially started in all the cats enrolled, with no improvement of vital parameters. Therefore, LFVR protocol was started. Conversely, HS administration alone or together with HES determined a rapid improvement of the vital parameters.

Cats received a mean±SD volume of 4.0±1.6 mL/kg of HS (range: 2-6 mL/kg) and 2.6±2.7 mL/kg of HES (range: 0-8 mL/kg). The administration of HS alone or together with HES determined an improvement of the vital parameters within 33±12.7 minutes, with a range of 15-60 minutes. In six patients (60%) it was sufficient a single administration of HS as the only solution (n=3/10) or in combination with HES (n=3/10). In the other four cases (40%) it was necessary to use a second bolus of HS, alone (n=2/10) or in combination with HES (n=2/10), before achieving the stabilization of the vital parameters (Table 3 and Figure 1).

Due to the severity of clinical condition and the small size of some of the patients, only in four cats (cases 3, 4, 5 and 9) it was possible to perform venous catheterization and only in two of them (cases 4 and 5) a blood sample suitable for the examinations was collected. In both cats, blood parameters showed the same trend: a slight decrease in PCV (T0: 25.5±9.5; T60: 24.0±10.0%), TS (T0: 62.5±7.5; T60: 59.0±9.0 g/L) and glucose values (T0: 12.7±4.9; T30: 11.2±3.8; T60: 10.3±3.3 mmol/L), and a remarkable reduction in lactate values (T0: 6.4±1.6; T30: 3.4±0.7; 1.75±0.9 mmol/L), suggesting the achievement of a good perfusion without an excessive haemodilution.

Table 2. Objective (rectal temperature [T°], heart rate [HR], respiratory rate [RR], and capillary refill time [CRT]) and subjective (quality of pulse, state of sensorium and mucous membrane colour) parameters at first clinical examination of the ten cats enrolled in the study

Case	Objective parameters				Subjective parameters		
	T° (°C)	*HR (bpm)*	*RR (breaths/min)*	*CRT (sec)*	*Pulse*	*State of sensorium*	*Mucous membrane colour*
1	34	120	40	2-3	Weak	Stuporous	Pale
2	30	46	28	>3	Weak	Stuporous	White
3	35.1	116	48	>3	Weak	Stuporous	White
4	28.9	120	40	>3	Weak	Stuporous	White
5	38.8	100	100	2-3	Weak	Depressed	Grey
6	36.8	94	80	2-3	Weak	Depressed	Grey
7	31.1	84	36	>3	Weak	Stuporous	Pale
8	29.8	88	56	2-3	Weak	Depressed	Pale
9	31.3	98	36	>3	Weak	Depressed	White
10	33	100	40	>3	Weak	Depressed	Pale
Mean	32.8	96.6	50.4				
±SD	3.3	21.9	22.6				
Median	32.2	99.0	40.0				

Table 3. Type of the shock, administration route (intravenous, IV; intraosseus, IO), volume of hypertonic saline solution (HS) and hydroxyethyl starch (HES), time until the initial and complete stabilization, follow up and outcome of the ten cats enrolled in the study

Case	**Type of shock**	**Route**	**Volume of fluid (mL/kg)**		**Time until stabilization (minutes)**		**Follow up**	
			HS	*HES*	*Beginning*	*Complete stabilization*	*Length*	*Outcome*
1	Distributive	IO/IV	6	4	5	45	25 months	Positive
2	Hypovolemic	IO	6	8	5	30	22 months	Positive
3	Hypovolemic	IV	4	4	5	30	3 days	Negative
4	Traumatic	IV	4	0	5	30	20 months	Positive
5	Traumatic	IV	2	0	5	15	17 months	Positive
6	Hypovolemic	IO	2	4	5	30	5 hours	Negative
7	Traumatic	IO	6	0	5	60	5 days	Negative
8	Traumatic	IO	2	0	5	30	5 days	Negative
9	Traumatic	IV	4	2	5	20	9 months	Positive
10	Hypovolemic	IO	4	4	5	40	6 months	Positive
Mean			4.0	2.6		33.0		
±SD			1.6	2.7		12.7		

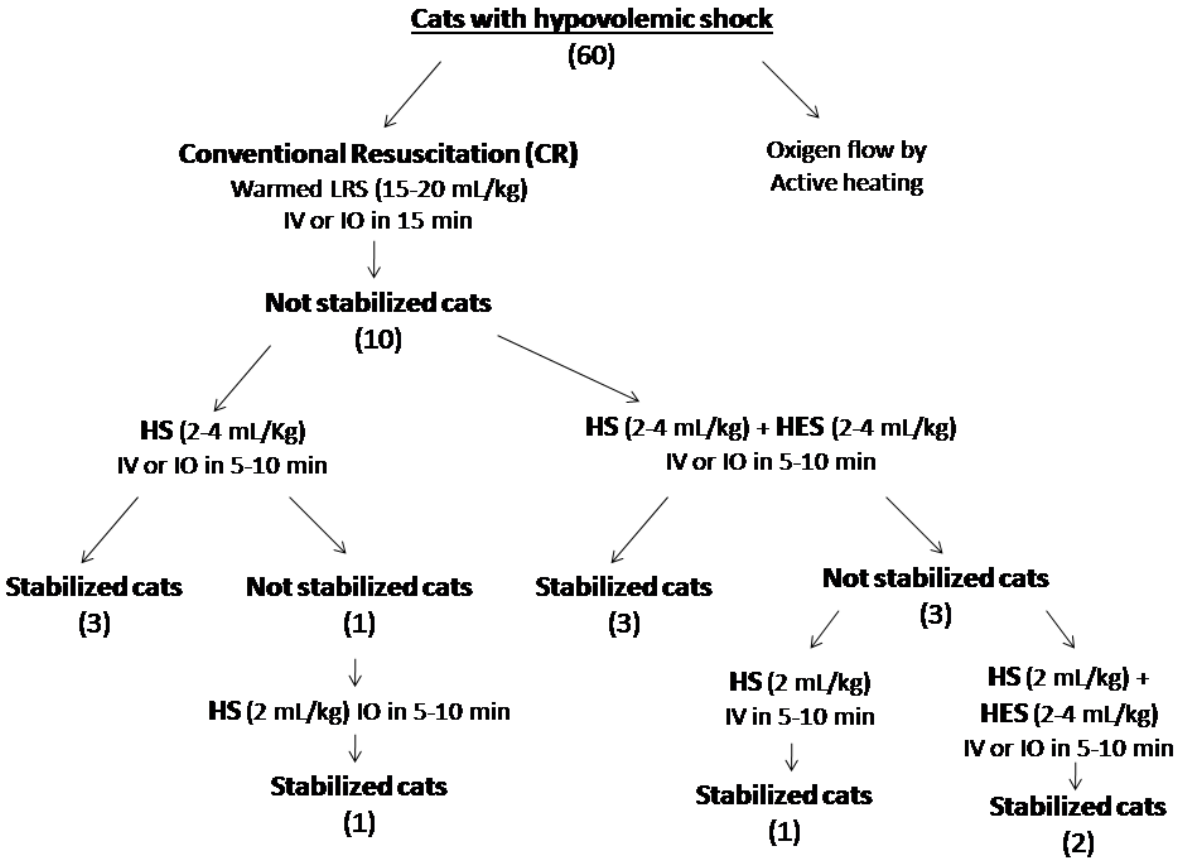
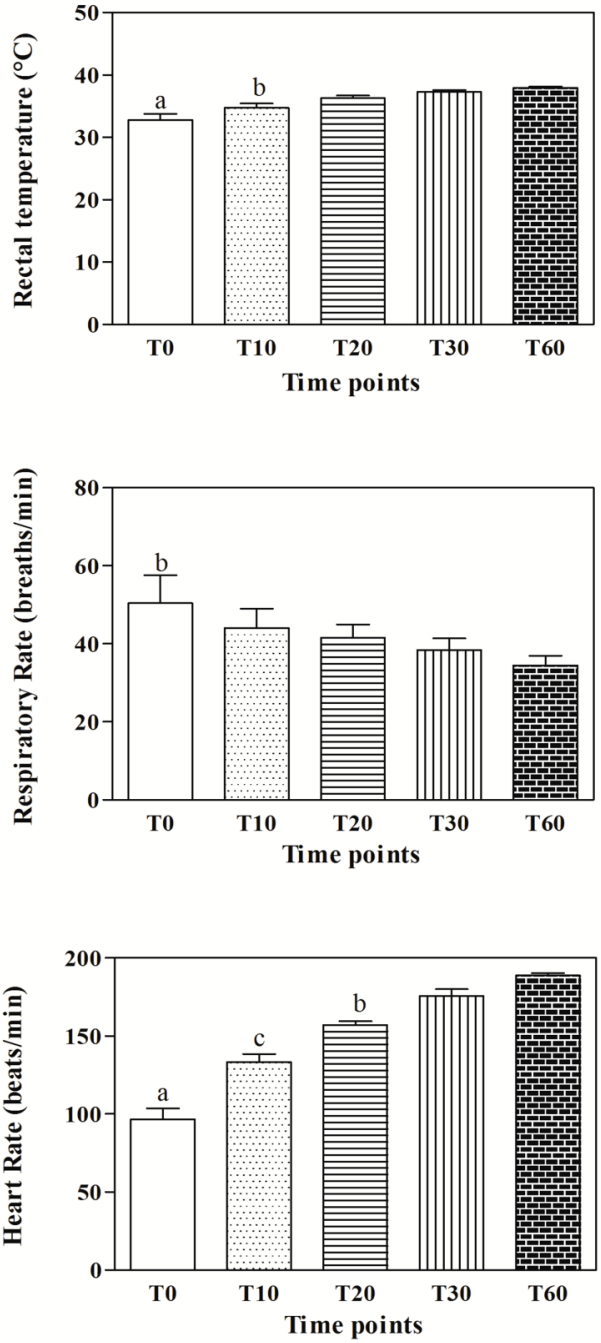


Figure 1. Limited volume fluid resuscitation (LFVR) algorithm in the 10 cats in non-cardiogenic shock enrolled in the study. LRS: Ringer's lactate solution; HS: hypertonic saline solution; HES: tetrastarch; IV: intravenous route; IO: intraosseus route

As reported in Figure 2, the statistical analysis showed a significant effect of LFVR ($p<0.001$) on T° ($p<0.0001$), HR ($p<0.0001$) and RR ($p<0.001$). In particular, lower values of T° were found at T0 compared to T10 ($p=0.03$), T20 ($p<0.0001$), T30 ($p<0.0001$) and T60 ($p<0.0001$) and at T10 compared to T30 ($p=0.002$) and T60 ($p<0.0001$). Regarding HR, the lowest values were found at T0 respect to T10 ($p<0.0001$), T20 ($p<0.0001$), T30 ($p<0.0001$) and T60 ($p<0.0001$), at T10 respect to T20 ($p=0.001$), T30 ($p<0.0001$) and T60 ($p<0.0001$) and at T20 respect to T30 ($p=0.01$) and T60 ($p<0.0001$). Finally, higher values of RR were found at T0 *versus* T30 ($p=0.01$) and T60 ($p<0.001$). For the small number of the data collected, statistical evaluations on blood parameters were not performed.

According to the patient's medical history and post-stabilization examinations, it was possible to establish the type and the origin of the shock in all the 10 cats: distributive, post-anaesthesia for sterilization surgery (case 1); hypovolemic, for severe dehydration (8-10%) due to rhinotracheitis and intestinal parasites (cases 2, 6 and 10) or renal failure and chronic enteritis (case 3); traumatic due to car accident (cases 4, 8 and 9), falling from heights (case 7) or aggression by dogs (case 5) (Table 3).

Of the ten cats, six (cases 1, 2, 4, 5, 9 and 10) currently enjoy good health (follow-up 6 to 25 months), whereas four died during hospitalization. Of these, one patient (case 3) died on the third day, because of worsening of the underlying disease (chronic renal failure); another one (case 6) died after 5 hours for pulmonary oedema due to a fault in the infusion pump and the last two (cases 7 and 8) died after 5 days for pneumonitis.



Significances (P<0.001): [a] vs T10, T20, T30 and T60; [b] vs T30 and T60; [c] vs T20, T30 and T60

Figure 2. Effect of LFVR protocol on mean ± standard deviation (SD) of rectal temperature, heart and respiratory rates values measured in the ten cats enrolled in the study. Relative significances throughout the experimental period time points (T0, before LFVR protocol and after the administration of the first bolus of Ringer Lactate solution, RLS; T10, T20, T30 and T60, after 10, 20, 30 and 60 minutes after LFVR administration, respectively)

1.1.4 Discussion

In the present study, the LVFR (hypertonic saline alone or associated with colloid) determined a rapid improvement of the vital parameters in contrast to the initial bolus of CR, suggesting a rapid improvement in blood perfusion and tissue oxygenation. No evident adverse effects occurred in any patient during the administration of the protocol.

Limited fluid volume resuscitation with hypertonic saline and colloid solutions expands plasma volume for 2-3 hours, quickly recalling the interstitial fluids and probably reducing endothelial permeability (Victorino et al., 2003). In contrast, in CR with isotonic crystalloids only 10-25% of fluids remains in the intravascular compartment after an hour from the administration (Silverstein et al., 2005). Further theoretical benefits of LFVR can be a reduced risk of hypothermia, damage from ischemia-reperfusion injury, interstitial oedema, possible with conventional resuscitation and coagulopathy by dilution (Hammond et al., 1014; Rocha-e-Silva and Poli de Figueiredo, 2005; Pascual et al., 2003). However, the real benefit on coagulation could be argued due to the good, if not even better, expansion of blood volume provided by LFVR.

Moreover, it has been demonstrated that the administration of HS together with dextran 70 has immunological and anti-inflammatory effects (Dubick et al., 2013) which last for 24 hours and could be useful in the prevention of multiple organ dysfunction syndrome (MODS) (Rizoli et al., 2006).

Only a few references reported the use of hypertonic saline solution in cats (Dupe et al., 1993; Muir and Sally, 1989). In an experimental model of hypovolemic shock, 4 mL/Kg intravenous administration of 7.5% NaCl solution determined positive hemodynamic effects, characterized by a rapid normalization of blood pressure, aortic flow and cardiac contractility and by the decreasing of peripheral resistance; the effect did not last more than 60 minutes (Muir and Sally, 1989).

However, LFVR protocols are not free from the risk of adverse effects. The hypertonic saline solution, besides being contraindicated in severe dehydration, can cause occasional premature ventricular contractions, bradyarrhythmias, transient hypotension and bronchoconstriction when administered at speeds faster than 1 mL/kg/min (Ford and Schaer, 1993; Stern, 2001). It can also determine transient hypernatremia, with alteration of sodium level in early hours after resuscitation, as well as affecting the osmolality and the levels of chloride, potassium and bicarbonate (Hammond et al., 2014). After administration of HS in peripheral vessels, it can be observed haemolysis which leads to haemoglobinuria (Muir and Sally, 1989). In addition, the HS can rapidly elevate cardiac output and blood pressure, which together with the increase of sodium level can increase the risk of congestive heart failure or neurological signs in patients with hyponatremia prior to the infusion (pontine myelinolysis) (Churcher et al., 1999). Although among the cats enrolled in this study four were clinically dehydrated, the LFVR protocol was started after the administration of the resuscitation bolus of isotonic crystalloids which partly improved the hydration status of the patients. Moreover, the rehydration fluid therapy was started immediately after the reanimation protocol.

Limited fluid volume resuscitation could also facilitate bleeding in the vascular injury site for clot breaking (re-bleeding) (Adamik et al., 2015). However, the bleeding is not a problem related only to hypertonic fluids, since it could be caused by all types of resuscitation fluid therapy.

In the present study, the administration of an initial bolus of crystalloid did not determine any improvement of the vital parameters, in contrast to the next administration of HS alone or associated with colloid. Conventional Resuscitation protocols usually consist of the administration of repeated boluses of isotonic crystalloids. However, cats seem less able to cope with large volumes of intravenous fluid and have been reported with a high incidence of pulmonary oedema, especially if they are hypothermic (Adamantos and Hughes, 2015). For this reason, and because the administration of the first bolus had determined no improvement

of the critical clinical conditions of the cats enrolled in this study, it was decided to start the LFVR as an alternative protocol. Approximately the 50% of haemodynamically unstable human patients treated with CR are non-responders, with no increase in cardiac output following intravenous fluid administration. This failed fluid challenge increase the risk of endothelial surface layer damage and tissue oedema (Cazzolli and Prittie, 2015). Although colloidal solutions have been reported with fairly common adverse effects, such as AKI and coagulopathy, in both human and veterinary medicine, these are commonly dose- and time-dependent. Many of the reports which describe these complications have been carried out in patients that have undergone a long period of treatment (Cazzolli and Prittie, 2015). On the contrary, some of the clinical trials on the use of HES within a shorter period for fluid resuscitation in patients with hypovolemic shock, found no evidences of an increased risk of AKI or coagulation impairment (Annane et al., 2015; Guidet et al., 2012). Moreover, in a recent study on rats with endotoxemia induced by lipopolysaccharide administration, fluid resuscitation with HES and hypertonic saline determined an amelioration of kidney injury (Wang et al., 2015).

Veterinary reports showed that bolus and continuous rate infusions of 6% hetastarch solution at moderate doses are well tolerated in feline and canine patients (Glover et al., 2014). Moreover, the administration of HES in cats did not results in a significant increase is serum creatinine compared with cats treated with crystalloid (Yozova et al., 2016). However, few safety and efficacy data exist in veterinary medicine on the use of HES as a resuscitation fluid.

In this study, the total administered volume of HES was far below (40%) the recommended daily dosage of 20 mL/kg/day (Mizzi et al. 2011), with an administered volume of 2.6±2.7 mL/kg and a maximum of 8 mL/kg. Regarding HTS, the total volume administered to each cat did not exceed the maximum range reported in the literature (Balakrishnan and Silverstein, 2015). Moreover, the fluid challenge has been immediately interrupted after the achievement

of the desired levels of vital signs and followed by a maintenance and correction of dehydration fluid therapy with isotonic crystalloids, so that the administration of HS and/or HES did not last for more than 60 minutes.

Response to treatment occurred very quickly, with the first positive effects already registered within 5 min after infusion. In particular, the increase in rectal temperature and heart rate occurred already after 10 min (T10), while the decrease of respiratory rate was significant only after 30 minutes (T30), suggesting an improvement in blood perfusion and, as a consequence, in tissue oxygenation.

In 30% of the patients a single administration of HS ± HES was sufficient, while in the other cases it was necessary to use one additional boluses of HS ± HES before reaching the stabilization of vital signs, always occurred within 15 and 60 minutes, even in very small (body weight <500 g) and immature (3-4 weeks old) cats.

The survival rate to the treatment was 90% on the first day and 60% on the fifth. Considering the severity of the clinical condition combined with the absence of response to the initial conventional resuscitation, the survival could be considered acceptable. Moreover, the cause of death was probably not to be attributed to a late adverse reaction to the treatment but rather to common complications in critically ill patients (e.g., pneumonitis) or irreversible chronic illness (chronic renal failure). In one case death was caused by infusion pump failure during hospitalization that quickly determined a fluid overload in a very young and small cat (1 month of age, 0.26 kg body weight).

The stabilization of vital parameters was optimal and persistent in nine of the ten cats enrolled in the study. Only in one case (case 7) it was observed a thermoregulatory control deficiency, probably due to a traumatic neurological damage.

The limitations of this study are the lack of a control group resuscitated only with crystalloids, the small size and the heterogeneity of the sample (cats were different for age, weight and cause of the shock) and the lack of electrolytes measurements before and after the

administration of the LFVR protocol. Therefore, further studies should be carried out on a wider number of animals, in order to establish the incidence of adverse effects, such as electrolytic abnormalities and eventual neurological signs related with an osmotic central damage induced by HS administration, as previously observed in hyponatremic patients (Churcher et al. 1999) and the effects of each specific LFVR protocol in order to establish if the administration of HES is really relevant.

The results obtained in this study seem to suggest that the administration of HS with or without HES could be used as an alternative fluid treatment in cats with severe non-cardiogenic shock. Although the present study should be considered a preliminary investigation due to the small number of cats enrolled, the absence of evident adverse effects, the good results achieved in the short term and the cost reduction due to a faster stabilization should support further investigations on LFVR effectiveness and safety in companion animals.

1.2 Clinical and therapeutic investigations on pericardial effusion in dogs

Pericardial effusion (PE) is an abnormal accumulation of fluid within the pericardial space and it represents the most common disease of the pericardium in dogs. Prevalence has been reported to be 0.43% and it was found in approximately 7% of dogs presented with clinical signs of cardiac disease (MacDonald et al., 2009). Most commonly, the aetiology of pericardial effusion in dogs is neoplastic or idiopathic in origin. The most common neoplastic causes of PE in dogs include haemangiosarcoma, heart base tumours (chemodectoma) or mesothelioma (Stafford et al., 2004; Shaw and Rush, 2007a). The idiopathic pericardial effusion (IPE) refers to a sterile hemorrhagic effusion, with no known aetiology. Prognosis of dogs with PE is strongly related to the cause of the effusion. Neoplastic PE, especially due to haemangiosarcoma, is associated with short survival times, ranging from 26 to 56 days, while IPE usually has a good long-term prognosis, ranging from 790 to 1068 days (Weisse et al., 2005; Stafford et al., 2004), although recurrent effusions might need a pericardiectomy in order to control clinical signs (Fine et al., 2003). Moreover, dogs with PE due to a heart base mass usually have a better prognosis than dogs with PE due to haemangiosarcoma (Weisse et al., 2005; Stafford et al., 2004).

The accumulation of fluid of any type in the pericardial space reduces diastolic cardiac filling and may lead to right heart failure; when the intra-pericardial pressure increases to a level higher than or equal to that of the right ventricle, cardiac tamponade occurs (Humm et al., 2009). However, patients with mild PE may not show clinical signs. The intra-pericardial pressure depends on the amount and on the rate of fluid accumulation. For this reason, even large volumes of effusion that accumulate slowly may result only in slight hemodynamic abnormalities, while small but rapid accumulation of pericardial effusion, as seen in acute neoplastic bleeding, may result in dramatic clinical presentation.

The most common findings on general clinical examination in dogs with PE are collapse or weakness, muffled heart sounds on heart auscultation, distended jugular veins, the presence of a jugular pulse and *pulsus paradoxus* and abdominal distension due to hepatomegaly and ascites. Vomiting has been described in association with PE in humans (Jabr, 2004). In a recent study in veterinary medicine, Fahey et al. (2017) described the occurrence of vomiting in the 51% of dogs with PE, especially in those with evidence of hypoperfusion.

The diagnosis of PE may be suspected based on the history, clinical examination and by the radiographic findings of a globoid heart silhouette, pleural effusion and enlargement of the caudal vena cava. However, echocardiography is the gold standard to confirm the diagnosis of PE and to distinguish between the non-neoplastic and neoplastic aetiologies, as well as to define the specific causes and locations of the neoplasm (MacDonald et al., 2009). Several echocardiographic views of the heart, such as right parasternal short-axis and long-axis views, left apical view and left cranial parasternal long-axis view, have to be performed in order to obtain a complete image of the heart and to detect any eventual masses, especially the ones localized in the right atrium (MacDonald et al., 2009). Cardiac tamponade is diagnosed when diastolic collapse of the right atrium, right ventricle or both is found together with pericardial effusion. In a study in 107 dogs, echocardiography showed a sensitivity and specificity of 82% and 100%, respectively, for the diagnosis of a cardiac mass in dogs with pericardial effusion. Moreover, it showed a high sensitivity and specificity in differentiating the specific type of cardiac mass (MacDonald et al., 2009). In this study, the two most common causes of pericardial effusion were haemangiosarcoma (33.6%) and idiopathic pericarditis (19.6%), but even mesothelioma (14%) and chemodectoma (12%) were fairly described. Recently, the diaphragmatic-hepatic (DH) view of the abdominal and thoracic focused assessment with sonography (AFAST and TFAST) has been described to be clinically helpful in detecting PE (Lisciandro, 2016), but not in distinguishing its specific cause.

Pericardiocentesis is required for the relief of cardiac tamponade and clinical sings in emergency conditions and to carry out cytological examinations of the fluid for diagnostic evaluations. In order to avoid the laceration of the left extramural coronary artery and to go through the cardiac notch of the right lung lobes, pericardiocentesis is usually performed with the dog in sternal or left lateral recumbency from the right haemithorax, in a small area where the lungs do not cover the heart and the pericardium is just beside the thoracic wall (Gidlewsky and Petrie, 2005). Echocardiography is helpful in finding the best intercostals space but, if it is not available, pericardiocentesis should be performed at the level of the cardiac notch, starting from the fourth or fifth interscostal space, dorsally to the costochondral junction. Several types of over- or through-the-needle catheters may be used to perform pericardiocentesis (Shaw and Rush, 2007b; Gidlewsky and Petrie, 2005). The most common adverse events reported during or following pericardiocentesis are cardiac puncture, arrhythmias, laceration of the tumour or the coronary artery, leading to intrapericardial haemorrhage or cardiopulmonary arrest, with a rate of occurrence of 15% in dogs (Humm et al., 2009). The rate of relapse of the fluid accumulation described in dogs both with or without neoplasia is high (Stafford et al., 2004), so repeated pericardiocentesis may be required.

In cases of recurrent PE, pericardiectomy is required to achieve a definitive diagnosis and to solve the problem of frequent relapses. Moreover, prolonged survival after pericardiectomy has been described in dogs with IPE, while dogs with neoplastic pericardial effusion have been reported to have shorter survival times (Stafford et al., 2004).

1.2.1 Aim of the study

The aim of this retrospective study was to evaluate the signalment, clinical signs, echocardiographic findings and epidemiology of PE in canine patients. Secondary objectives included defining the specific causes of PE and locations of tumours within the heart and

determining the clinical usefulness of a new pericardiocentesis technique, using a "*fistula needle*" for haemodialysis.

1.2.2 Materials and method

Case selection criteria

A database of dogs referred for specialist cardiology and echocardiographic examination from 2009 to 2016 was reviewed. The examinations were performed in a high standard veterinary referral clinic in Sicily (Italy), on client-owned dogs, after the informed consent had been signed by the owners.

Animal husbandry and clinical procedures were in accordance with the standards recommended by the *Guide for the Care and Use of Laboratory Animals* and Directive 2010/63/EU on the protection of animals used for scientific purposes.

Diagnostic tests

All the dogs were subjected to a general physical examination, radiographs of the thorax in dorso-ventral and right lateral recumbency and echocardiography in right parasternal short-axis and long-axis views, left apical view and left cranial parasternal long-axis view. All the echocardiographic examinations were performed by the same specialized operator by mean of an ultrasound machine (Esaote My Lab 30 Gold, Genova, Italy) with a multi-frequency probe of 2.5-3.5 MHz with sterile cover.

Pericardiocentesis

When required, an echo-guided pericardiocentesis was performed. The right lateral thorax was clipped and aseptically prepared between the second and the eight intercostal spaces. Patients were placed in right lateral recumbency, on an echocardiography table with a suitable hole for performing the diagnostics procedures. Pericardiocentesis was performed between the fourth and the fifth intercostal space by mean of a 17 gauge "*fistula needle*" (Fresenius

Medical Care, Bad Homburg, Germany) (Figure 1 and 2), commonly used for haemodialysis, with a catheter of 2.5 cm provided with a lateral hole and two wings, connected to a flexible tube 30 cm long. A large 50 mL syringe and a three-way stop cock were attached to it (Figure 3). Following the echo-guided insertion of the needle into the intercostal space and the perforation of the pericardium, the aspiration of the effusion was performed. A sample of the effusion was kept for physical and cytological examinations in blood tubes and stored under refrigeration until examinations were performed. During the pericardiocentesis procedure, continuous electrocardiogram monitoring was performed. Complete echocardiography was repeated at the end of the procedure, in order to detect any mass or abnormality that may have been hidden by the effusion.

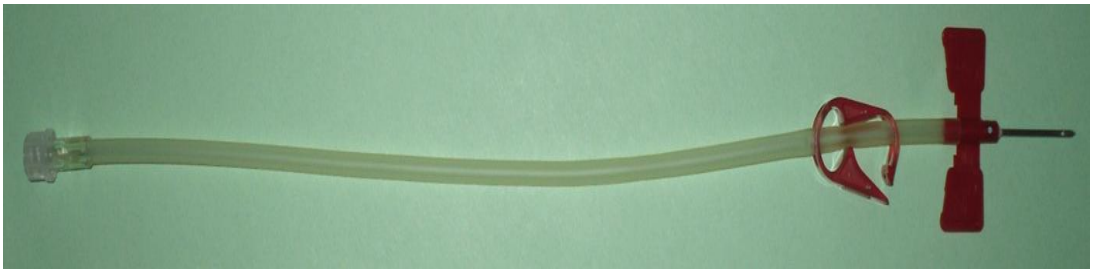


Figure 1. "*Fistula needle*" (Fresenius Medical Care, Bad Homburg, Germany) used for the pericardiocentesis

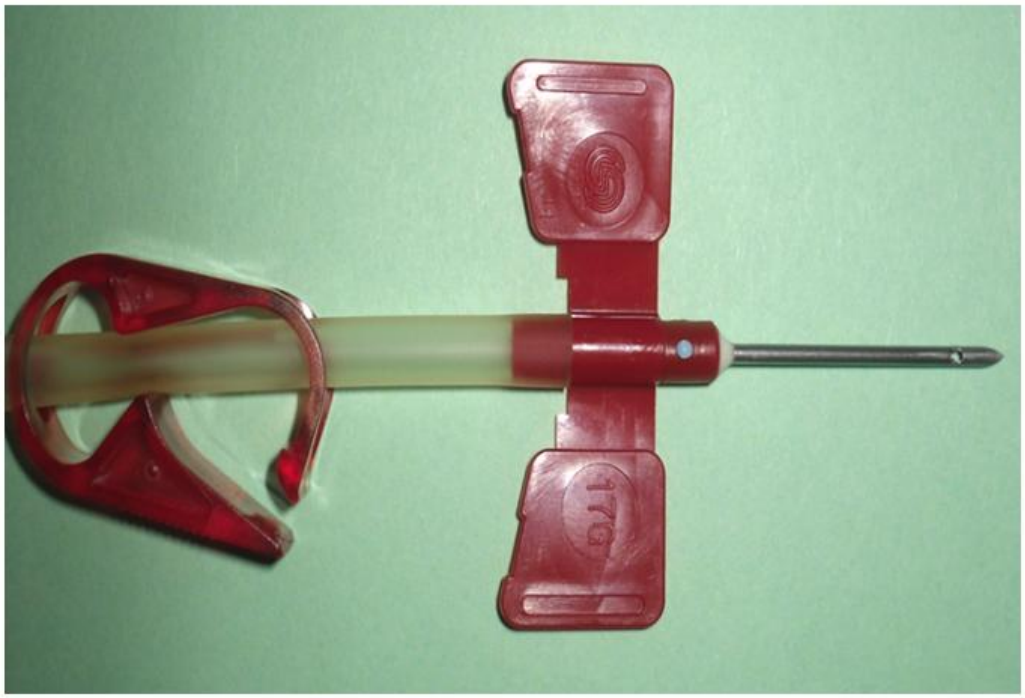


Figure 2. Detail of the tip of the "*fistula needle*"

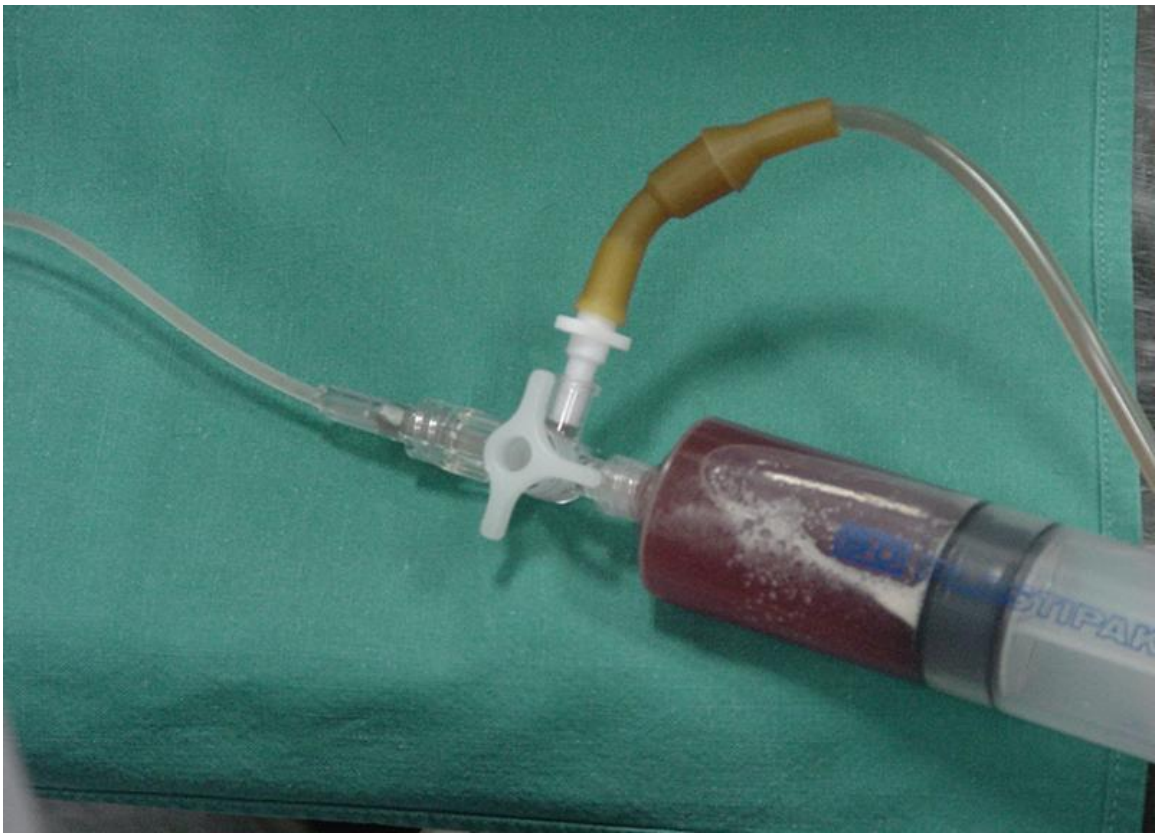


Figure 3. Three-way stop cock and 50 mL syringe attached to the "*fistula needle*". In the picture is visible a sample of the pericardial effusion collected during the pericardiocentesis

Statistical analysis

A descriptive statistical analysis (percentage, mean, standard deviation, SD, and range) was applied for the age and gender for each breed of dog enrolled in the study. Percentages have been calculated for the incidence rate of pericardial effusion, specific cause of PE and cardiac tamponade in the dogs enrolled in the study. Data were analyzed using a commercially available statistical software program (STATISTICA 7, Stat Soft Inc., USA, 2003).

1.2.3 Results

The records of 5304 dogs referred for specialist cardiology and echocardiographic examination were reviewed for this study.

Dogs were of different breeds, age, gender, type and severity of the cardiac disease. The total number of dogs found with pericardial effusion was 91 (1.7%). Among this, 20 were female (21.98%) and 71 were male (78.02%). The mean age at presentation was 10.63±2.90 standard deviation (SD) years (ranging from 1 to 19 years). Breed, gender and age of the 91 dogs with PE included in the study are reported in Table 1.

Table 1. Number (n) of animal, gender (female [F] and male [M]) and descriptive statistic of ages (Mean, Standard Deviation [SD], minimum [Min] and maximum [Max] values) for each the breed of dogs with pericardial effusion (PE) enrolled in the study

Breed	***N***	***Gender***		***Age***			
		F (n)	***M (n)***	***Mean***	***SD***	***Min***	***Max***
Mongrel dog	38	7	31	11.74	2.45	6	19
German shepherd	5	0	5	9.8	0.84	9	11
Beagle	5	0	5	9.75	1.89	7	11
Labrador retriever	4	0	4	7.25	3.86	2	11
Miniature Poodle	4	0	4	12	1.63	10	14
Italian mastiff	4	1	3	8.5	3.11	5	12
Yorkshire terrier	3	1	2	11.33	1.53	10	13
Pit bull	3	2	1	11	3.46	9	15
English setter	3	2	1	9.33	4.72	4	13
Pug	2	0	2	8.5	0.71	8	9
Golden retriever	2	0	2	10	0	10	10
*CKCS**	2	1	1	8.5	0.71	8	9
Siberian husky	2	1	1	11.5	0.71	11	12
Irish setter	1	1	/	/	/	10	/
Pekingese	1	/	1	/	/	15	/
Rottweiler	1	/	1	/	/	8	/
German pinscher	1	1	/	/	/	12	/
Dogue de Bordeaux	1	1	/	/	/	1	/
Bull mastiff	1	1	/	/	/	8	/
Sharpei	1	1	/	/	/	9	/
Maltese	1	/	1	/	/	8	/
Etna Cirneco	1	/	1	/	/	15	/
Dachshund	1	/	1	/	/	6	/
Chihuahua	1	/	1	/	/	12	/
Hound	1	/	1	/	/	10	/
Shih Tzu	1	/	1	/	/	15	/
Newfoundland	1	/	1	/	/	8	/

**Cavalier King Charles Spaniel*

Among the 91 dogs with PE, cardiac tamponade, with signs of obstructive shock (lethargy, distended jugular veins, jugular pulse and *pulsus paradoxus*, tachycardia and weak peripheral pulse), occurred in 37 cases (40.65%). All the patients with pericardial effusion showed various degrees of ascites.

Radiographs of the thorax showed cardiomegaly with a globular cardiac silhouette in all the 91 dogs. On echocardiographic examination, clear evidence of a neoplasia was found in 33 cases (36.26%); among these, 15 were localised in the right atrium (45.45%) (Figure 4), 9 were heart base masses (27.27%) (Figure 5), 5 were on the left atrium (15.15%) and 4 were in the thorax (mediastinum and pleural space) (12.12%).

In 32 cases (35.16%) a severe mitral regurgitation together with tricuspid regurgitation was detected as the possible cause of the PE; among these, pulmonary hypertension was found in 22 cases (68.75%). In only one case (1.10%) the PE was due to a foreign body (a lead bullet) lodged in the interventricular septum (Figure 6). In the remaining 25 cases (27.47%) a clear aetiology was not found (Table 2).

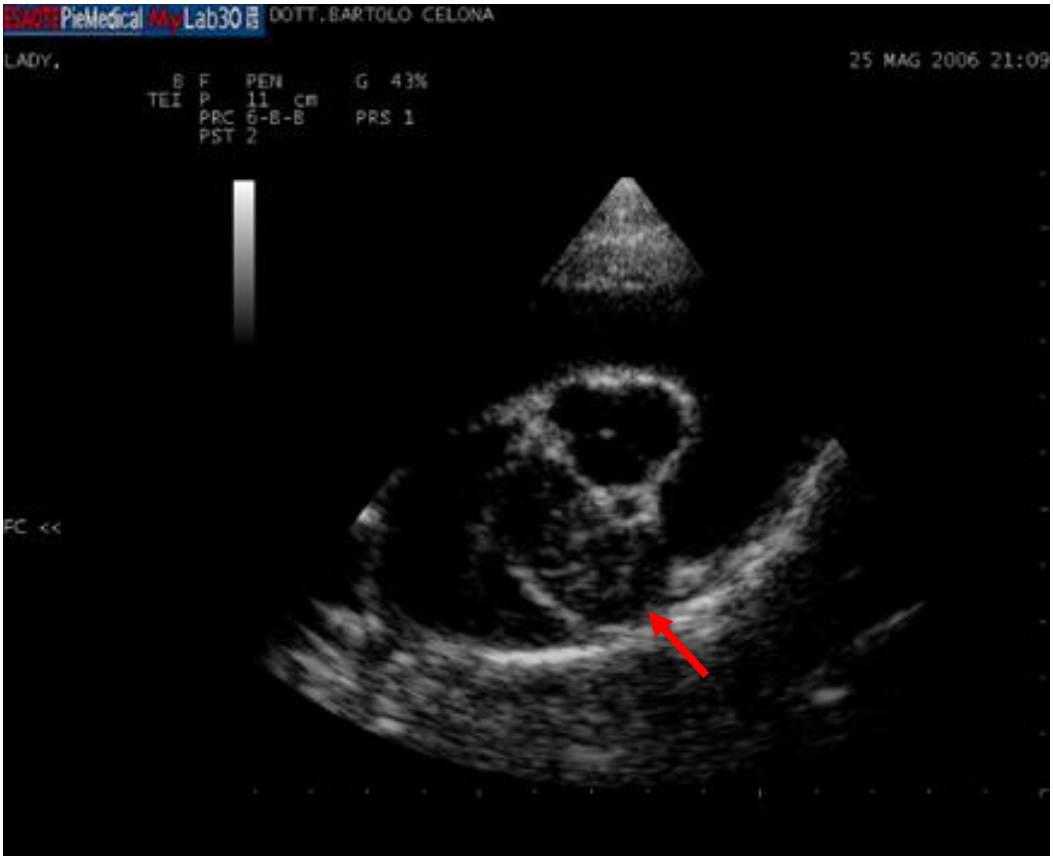


Figure 4. Ecocardiographic picture, in left apical view, of a right auricle neoplasia (arrow)

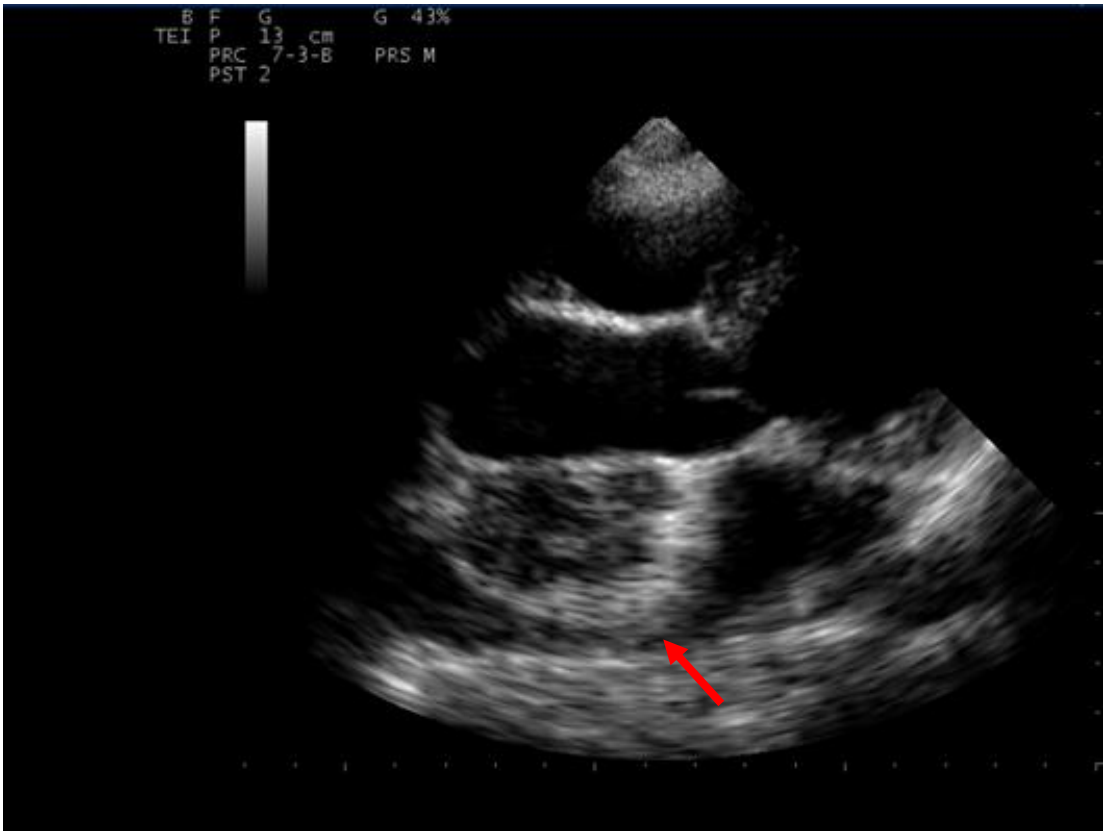


Figure 5. Ecocardiographic picture, in left apical view, of a heart base tumour (arrow)

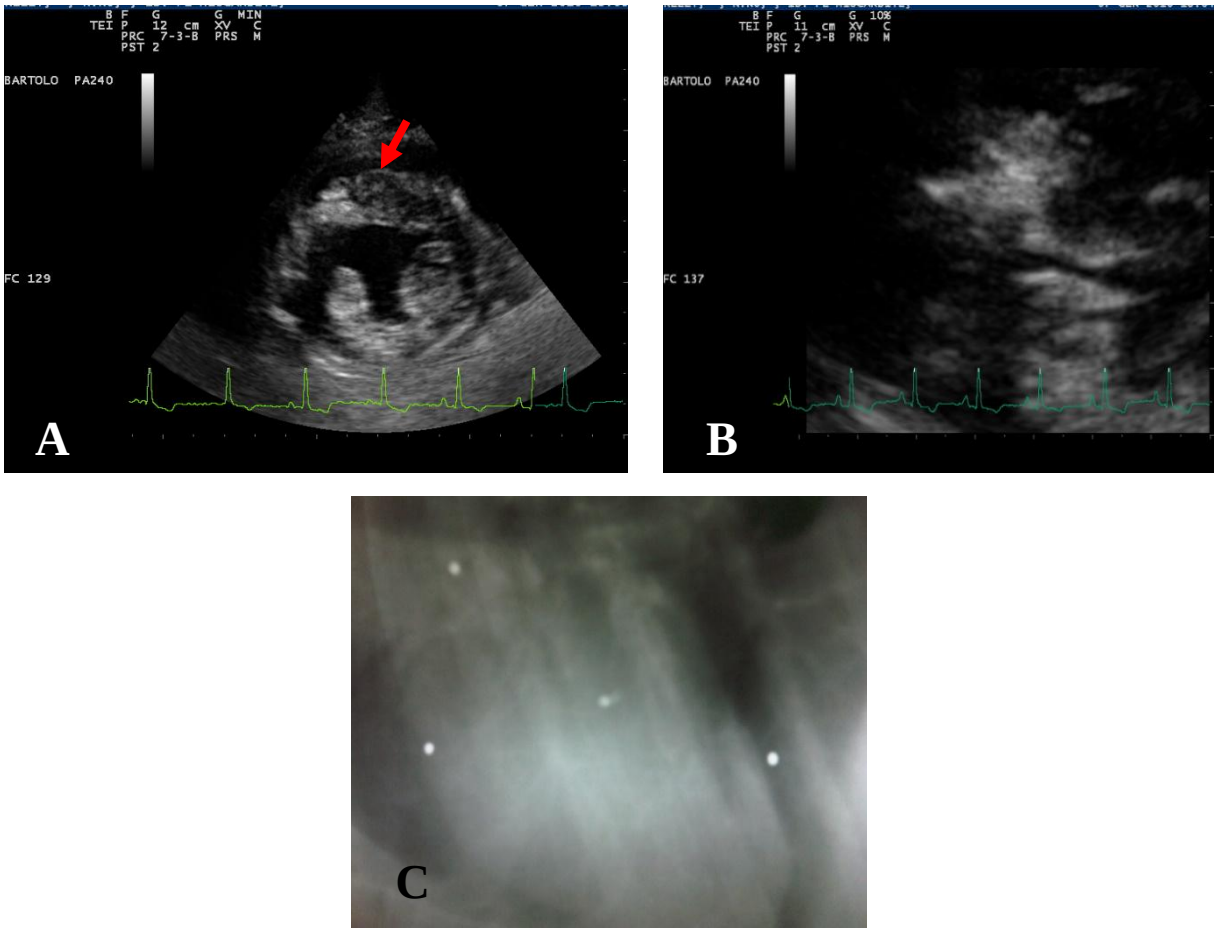


Figure 6. Ecocardiographic, in right parasternal short axis view (**A** and **B**), and radiologic (**C**) pictures of a foreign body (a lead bullet) stuck in the interventricular septum (arrow). In **B** an enlarged ecocardigraphic picture of the foreign body

Table 2. Incidence of tumours, valve regurgitation, foreign body and unidentified cause: number of dogs (n) and cardiac tamponade (CT) for each specific condition, for each breed of dogs with pericardial effusion (PE) enrolled in the study

Breed	***n***	***Aetiology***							
		Tumours		*Valve regurgitation*		*Foreign body*		*Unidentified cause*	
		n	*CT*	*N*	*CT*	*n*	*CT*	*N*	*CT*
Mongrel dog	38	16	7	16	0	0	0	6	3
German shepherd	5	2	0	1	0	0	0	2	2
Beagle	5	2	1	2	0	0	0	1	1
Labrador retriever	4	1	0	0	0	0	0	3	2
Miniature Poodle	4	0	0	4	0	0	0	0	0
Italian mastiff	4	2	2	0	0	0	0	2	2
Yorkshire terrier	3	2	1	0	0	0	0	1	1
Pit bull	3	0	0	0	0	0	0	3	3
English setter	3	0	0	1	0	1	0	1	1
Pug	2	0	0	0	0	0	0	2	1
Golden retriever	2	1	0	0	0	0	0	1	1
*CKCS**	2	0	0	2	0	0	0	0	0
Siberian husky	2	2	2	0	0	0	0	0	0
Irish setter	1	0	0	1	0	0	0	0	0
Pekingese	1	0	0	1	0	0	0	0	0
Rottweiler	1	0	0	0	0	0	0	1	1
German pinscher	1	0	0	0	0	0	0	1	1
Dogue de Bordeaux	1	1	1	0	0	0	0	0	0
Bull mastiff	1	1	1	0	0	0	0	0	0
Sharpei	1	0	0	0	0	0	0	1	1
Maltese	1	0	0	1	0	0	0	0	0
Etna Cirneco	1	0	0	1	0	0	0	0	0
Dachshund	1	0	0	1	0	0	0	0	0
Chihuahua	1	1	0	0	0	0	0	0	0
Hound	1	1	1	0	0	0	0	0	0
Shih Tzu	1	0	0	1	0	0	0	0	0
Newfoundland	1	1	1	0	0	0	0	0	0

**Cavalier King Charles Spaniel*

No cardiac tamponade was found in any of the patients with PE due to severe mitral regurgitation.

Echo-guided pericardiocentesis was performed in 28 cases (30.77% of dogs with PE) with cardiac tamponade (73.68% of patients with cardiac tamponade). In all animals before and after the execution of the pericardiocentesis, a complete echocardiographic examination was performed. No patient required sedation or general anaesthesia. The remaining nine patients with cardiac tamponade, for which pericardiocentesis was not performed, were euthanized immediately after the initial diagnosis at the will of the owner, because of the severity of the clinical condition combined with a poor prognosis.

Pericardiocentesis was carried out until the complete disappearance of the echocardiographic signs of cardiac tamponade (normal distension of the cardiac chambers) and, at least, until almost complete aspiration of the effusion (Figure 7). The procedure lasted in all cases no more than 15 minutes.

In all patients that underwent pericardiocentesis, no adverse effects or complications occurred, except for a few cases of occasional ventricular ectopies during the final stage of the procedure, due to the contact between the tip of the needle with the free wall of the right ventricle. All patients were discharged the same day, with an antibiotic and analgesic therapy for 5 and 2 days, respectively. All the patients experienced an immediate improvement of their clinical condition with gradual disappearance of *pulsus paradoxus*, jugular veins distension and jugular pulse, improvement of the mentation state and of the quality of the peripheral pulse, a normalization in heart rate, and, afterwards, reduction of the ascites. No adverse effects or complications following pericardicentesis were noticed in any of the patients during the 48 hours of follow up.

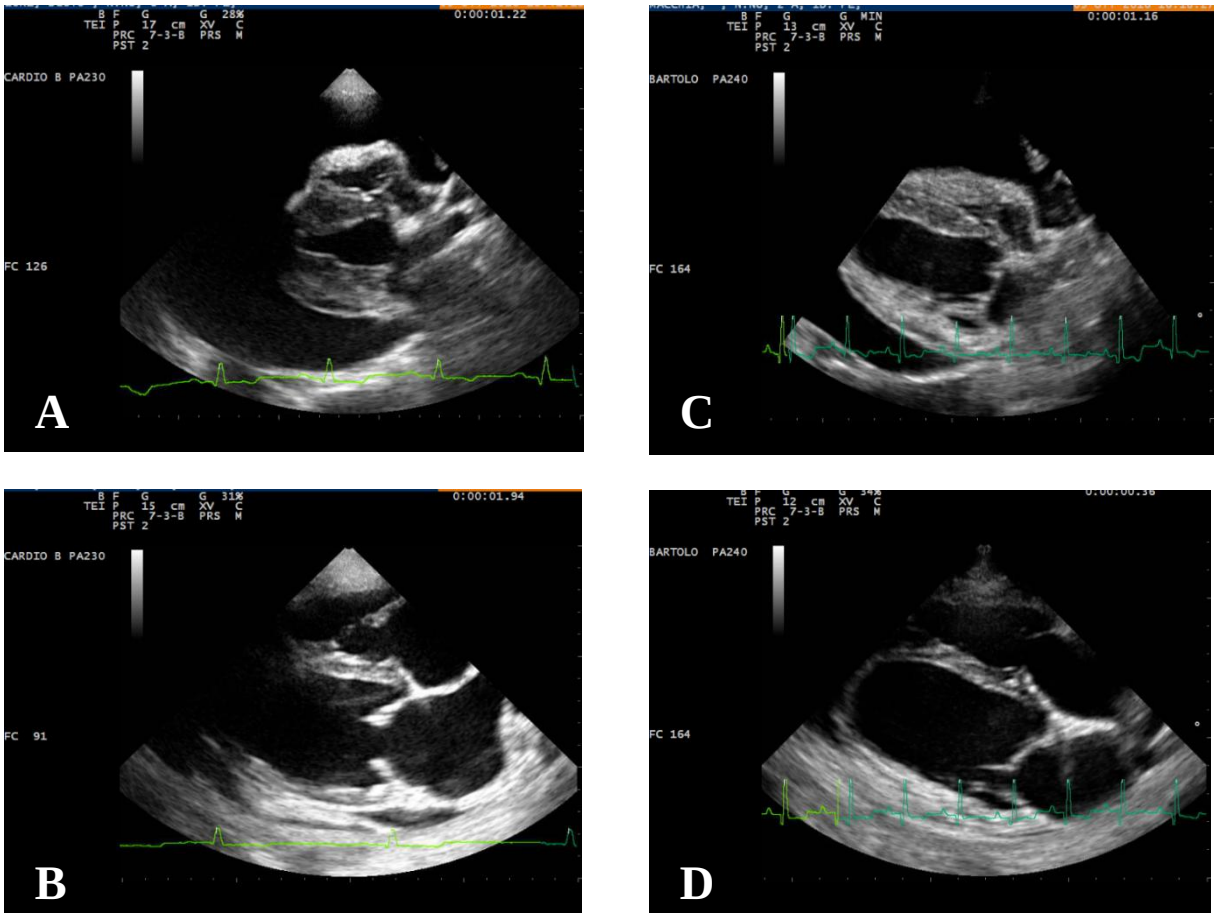


Figure 7. Ecocardiographic pictures in right parasternal long axis view, of two dogs with cardiac tamponade due to pericardial effusion, before (**A** and **C**) and after (**B** and **D**, respectively) pericardiocentesis. Before pericardiocentesis (**A** and **C**), it can be noticed the collapsed right atrium and a large amount of pericardial effusion. After pericardiocentesis (**B** and **D**), the right atrium and ventricle are completely distended with only a slight amount of pericardial effusion left

1.2.4 Discussion

Pericardial effusion is reported to be a fairly common disease in dogs due to several different causes and the prognosis varies from good to grave depending on the aetiology of the pericardial effusion (MacDonald et al., 2009). Most commonly, pericardial effusion in the dog has a neoplastic aetiology or is idiopathic. Less common reported causes of PE are infectious diseases (Ribas et al., 2015; Johnson et al., 2003; Shubitz et al., 2001; Aronson and Gregory, 1995), coagulopathies (Petrus and Henik, 1999), foreign bodies (Kolm et al., 2001), trauma (Witt and Mathews, 2000), congenital defects (Evans and Bierry, 1980), uremic pericarditis, left atrial rupture (Sadanaga et al., 1990), right-sided heart failure (Shaw and Rush, 2007a) and chronic mitral degenerative valve disease (DVD), secondary to myxomatous degeneration (Chetboul and Tissier, 2012).

The results obtained in this retrospective study showed that the most common causes of PE were tumours and chronic DVD, such as the myxomatous degeneration of the mitral and tricuspid valves.

Regarding heart tumours, our results are consistent with the ones found in other studies (MacDonald et al., 2009; Stafford et al., 2004).

In literature, the second cause reported of PE is idiopathic pericarditis (Shaw and Rush, 2007a; Stafford et al., 2004) that, together with tumours, represents more than 90% of the causes of PE. The degenerative mitral valve disease is a well-known, although unusual, cause of PE (Mellanby et al., 2002). On the contrary, among the dogs with PE enrolled in this retrospective study, mitral and tricuspid valves degeneration was the most common non-neoplastic cause of PE. Therefore, according to this finding, the DVD should be considered as a possible and not such unusual cause of PE in the dog.

Moreover, in this study patients with PE were more likely to be male (78.02%) instead of female (21.98%). This can be due to the fact that the incidence of DVD is higher among male dogs, as reported in the literature (Borgarelli et al., 2012).

Cardiac tamponade, a common PE complication, is a real emergency in both human and veterinary medicine, causing a serious hemodynamic deficit, which, if not promptly treated, can even cause the death of the patient. For this reason, diagnosis and treatment of cardiac tamponade have to be carried out as quickly as possible in order to save the patient's life (Gidlewsky and Petrie, 2005).

Clinical and radiographic examinations of veterinary patients could provide a strong suspicion of cardiac tamponade. However, echocardiography is the gold standard for the diagnosis of PE and cardiac tamponade; moreover, it is extremely useful in the execution of pericardiocentesis (MacDonald et al., 2009).

In this study, the use of a *fistula needle* for haemodialysis seemed to be an inexpensive, safe and effective procedure in dogs with PE without adverse effects after a follow up of 48 hours. This device, characterized by a lateral hole and a fairly large diameter (17G) but a relatively short cannula (2.5 cm), seemed to be less traumatic than the commercial kit available for pericardiocentesis, allowing the procedure to be carried out without the need of a sedation or general anaesthesia that could be risky in patients with a severe hemodynamic failure. In all the patients, the device used was able to drain almost the whole amount of pericardial effusion, or at least the amount needed to determine the complete disappearance of the echocardiographic signs of cardiac tamponade (normal distension of the cardiac chambers).

In a previous study, percentages of adverse effects (dysarrhythmias, cardiopulmonary arrest, bleeding into the pericardium, ventricular tachycardia and atrial fibrillation) of 10.7% and 15.2% within 1 hour and 48 hours, respectively, after the end of the pericardiocentesis, have been described, and the 41% of dogs died or were euthanized within the first 48 hours (Humm et al., 2009). This can be due to the different device used, but it may also be due to the fact that, in the mentioned study, pericardiocentesis was not echo-guided. The most common adverse effects reported were arrhythmias, cardiopulmonary arrest, continued bleeding into

the pericardium due to coronary artery laceration, ventricular tachycardia and atrial fibrillation due to heart puncture (Humm et al., 2009; Gidlewsky and Petrie, 2005).

Pericardial effusion is an emergency condition that requires a prompt diagnosis and treatment. Echocardiography often makes possible to achieve a causal diagnosis, with the relative prognosis. Moreover, it ensures the best and safest approach to pericardiocentesis that, if properly executed, could be lifesaving in case of cardiac tamponade.

This retrospective study showed the relatively high incidence of PE due to a severe bilateral valve regurgitation in adult to elderly dogs of different breeds, previously reported to be uncommon. The pathophysiology of pericardial effusion in human and veterinary patients with mitral and/or tricuspid valve degeneration has been associated with two different mechanisms. Left atrial rupture is an infrequent complication of canine mitral valve disease (MDV). The enlargement of the left atrium (LA) caused by the mitral regurgitation, determines an increase in LA pressure and mechanical trauma of the atrial endocardium. These conditions may determine the occurrence of endocardial splitting of the caudal wall of the LA which can cause pericardial effusion, characterised by the presence of a laminated blood clot in the pericardial space (Chetboul and Tissier, 2012). Although no clear atrial rupture has been detected in any of the dogs with PE associated with mitral valve degeneration enrolled in this study, it is not possible to rule out that small splitting could have been missed on echocardiography. Congestion is the second mechanism that could cause pericardial effusion in patient with congestive heart failure (CHF) due to degenerative valve disease. The accumulation of fluid caused by the CHF can occur in all the body cavity, included the pericardial space (Kataoka, 2000). This was, most likely, the mechanism behind PE in most of the dogs enrolled in this study.

In this retrospective study, none of the patients with PE due to degenerative valve disease showed clinical and echocardiophic signs of cardiac tamponade and obstructive shock. This can be due to the chronicity and slow progression of the disease, so that the slight progressive

accumulation of fluid into the pericardial space was slow enough to determine the onset of the compensatory mechanisms. Finally, the high incidence of PE due to degenerative valve disease reported in this study can be related to a possible inherent bias of case selection as all the dogs enrolled in the study were referred for a specialist cardiologic and echocardiographic examinations. However, also in the retrospective study of Johnson et al., (2004), the dogs were referred to cardiac centres for specialist examinations.

The use of a "*fistula needle*" for haemodialysis as a new device for pericardiocentesis in dogs showed no adverse effects compared to other techniques previously described. Unfortunately, considering that the dogs were referred for diagnostic reason and, after the end of the pericardiocentesis, came back to the referring veterinarians, information regarding the eventual onset of side effects and relapses in the medium/long-term follow-up are lacking. For this reason, further studies should be carried out on a larger number of dogs in order to exclude the possibility of complications onset.

II. RESPIRATORY SYSTEM

2.1 Autologous blood transfusion (ABT) in a puppy with haemothorax due to anticoagulant rodenticide intoxication

Dicumarine rodentice intoxication

Dicumarine rodenticides are pesticides, derived from dicumarol, with anticoagulant properties used against rodents. The first generation of rodenticides was created during 1940s, and they were mainly represented by the warfarin and coumarin. However, with time, most of the rodents became resistant to warfarin, and new generations of rodenticides were created. The second generation includes brodifacoum, difethialone, difenacoum and bromadiolone, while the third includes chlorophacinone, diphacinone and pindone. Dicumarine rodenticides are well absorbed after oral ingestion and the plasmatic peak usually occurs within 12 hours. They have high binding affinity with plasmatic proteins, are metabolized by the liver and excreted through the urine. Moreover, some rodenticides may undergo an entero-hepatic recirculation (Petterino et al., 2004). The plasmatic half-life is longer for the newer generation and the duration of action goes from 14 days for the warfarin to 30 days for the brodifacoum.
The mechanism of activity is based on the inhibition of the vitamin K epoxide reductase. Vitamin K-dependent clotting factors (II, VII, IX and X) require vitamin K (hydroquinone) for their activation, which leads to the oxidation of reduced vitamin K to inactive epoxide. The enzyme vitamin K epoxide reductase catalyzes the conversion of inactive epoxide back to active hydroquinone. Consequently, the inhibition of this enzyme caused by dicumarine rodenticides, causes a block of the extrinsic, intrinsic and common ways and the development of a secondary coagulopathy.

Clinical signs occur from 2 to 10 days after the exposition to the toxin, because circulating factors remain active until their degradation. The most common clinical signs reported with dicumarine poisoning are lethargy, exercise intolerance, dyspnoea (due to haemothorax or to lung haemorrhage) (Binev et al., 2005), lameness (due to haemarthrosis), haematochezia or melena, haematemesis and haematuria. On general physical examination, pale mucous membranes and mucosal or intracavitary haemorrhage can be found. The most common location for haemorrhage in the dog are the subcutaneous tissue, body cavities (thorax, abdomen and pericardium) (Park et al., 2011) and the bladder. Fatal haemorrhage in the central nervous system (CNS) may also occur.

Laboratory findings usually reported with rodenticides poisoning are prolonged clotting times (prothrombin time, PT, and activated partial thromboplastin time, aPTT, with normal fibrinogen) and normocytic normochromic anaemia. The PT is the first to become abnormal because the factor VII is the one with the shortest half life (6.2 hours). In fact, anticoagulant rodenticides, by antagonizing the vitamin K epoxide reductase, cause a depletion of the body's store active vitamin K-dependent factors II, VII, IX and X. The PT is dependent on factor VII, so that is why this parameter is the first to be affected in dogs with dicumarine rodenticides intoxication. Factors II, IX and X (intrinsic pathway) values are expressed by aPTT and have longer half life compared to factor VII, so that the alteration of the aPTT level occurs only in a second moment. However, when bleeding occurs, usually all the factors are already depleted, and both coagulation times are abnormal (Waddell et al., 2013). The activated clotting time (ACT) reflects the intrinsic pathway and, therefore, will not be prolonged until factor depletion is severe. If PT is the only abnormal clotting time, or if its alterations are greater compared to the aPTT, dicumarine rodenticides intoxication is likely (Waddel et al., 2013). On the contrary, if aPTT is more severely prolonged than PT, the intoxication is unlikely.

The specific treatment for dicumarine rodenticides intoxication consists in the administration of vitamin K_1 (phytomenadione) at the dose of 5 mg/kg subcoutaneously (SC) or intravenously (IV), followed by 2.5 mg/kg two times per day orally (OS) for at least 28 days (Brown and Waddell, 2015). The intravenous route has been associated with a high frequency of anaphylaxis after the administration of vitamin K_1, so it should be avoided. Vitamin K_1 has a better availability when ingested, so that the therapy should be switched to orally as soon as possible. Two days after the last administration, clotting times have to be checked and, if still abnormal, the treatment has to be prolonged. However, before starting the specific treatment, severely affected animals may require emergency treatments for the replacement of clotting factors and the correction of the coagulopathy. Fresh plasma, fresh frozen plasma (10-20 mL/kg) and fresh whole blood are the only blood products able to provide active clotting factors, and they should be administered until the normalization of clotting times (Brown and Waddell, 2015). If severe anaemia occurs, patients may benefit of the administration of whole blood or packed red bloods cells. Autologous blood transfusion could also be performed in patients with severe anaemia in order to maintain the haematocrit above life-threatening levels (Brown and Waddell, 2015). If patients show severe respiratory distress due to a haemorrhagic pleural effusion, thoracocentesis could be perform immediately. However, thoracocentesis, as well as abdominocentesis and pericardiocentesis, ideally should be postponed after the correction of the coagulopathy, due to the risk of further worsening of the bleeding caused by the insertion even of a small needle, unless the severe clinical condition of the patient requires a prompt intervention. Supportive care, such as oxygen supply, are most of the time necessary until the anaemia and/or the respiratory distress are solved.

Autologous blood transfusion (ABT)

Autologous blood transfusion, also called autotransfusion, is defined as a specific type of blood transfusion where donor and recipient are the same individual. The first autotransfusion was performed in 1874 by James Highmore in women from *post-partum* blood losses

(Highmore, 1874). A few years later, the blood collected from an amputated leg was autotransfused to the same patient through the femoral vein (Duncan, 1886). After the discovery of human blood groups in 1990, by Karl Landsteiner, and of anticoagulants used for the blood storage, homologous blood transfusion gradually became more popular. However, especially during war periods, when it was more difficult to find blood or blood product, the autotransfusion gained a new interest.

Although its benefits have been known since more than one hundred years, the autologous blood transfusion has been used more constantly only since a few decades. In human medicine blood autotransfusion has been used since 1970s when it was applied to more 400 patients with traumatic haemothorax, without any significant complication (Symbas, 1978). In 1988, the autotrasfusion was performed in 18 patients with traumatic haemothorax by draining the blood in a sterile bag for blood transfusion of 750 mL, through a micro filter with pores of 120 µm (Barriot et al., 1988).

Over the years, different techniques of autotransfusion have been used in human medicine, classified as pre-operative, intra-operative or post-operative. The pre-operative autotransfusion consists in the collection of a certain amount of blood, based on body weight (usually 6 mL/kg), from a patient that will undergo a surgical procedure within 2 to 3 weeks, and anyway not after one week before the surgery. The blood collected can be stored at 4° C in bags with different type of anticoagulant, like citrate phosphate dextroseadenine (CPDA), for 35 days, and acid citrate dextrose (ACD) or saline adenine glucose-mannitol (SAG-M) for 42 days.

Among intra-operative autotransfusion techniques, Normovolemic Acute Haemodilution (NAH) consists in collecting the blood immediately before the beginning of the surgery to be re-administered during the surgery or immediately after (within 8 hours after the collection), if the surgery is supposed to cause a severe bleeding. The amount of blood is immediately replaced with the same amount of NaCl 0.9% in order to maintain the euvolemia. Another

possibility of intra-operative autotransfusion, is the collection of blood from the surgical area which is re-administered with or without blood washing and concentration of red blood cells. The last type of autotransfusion, the peri-operative technique, consist in the collection of blood coming out from drains, which is collected in sterile bags, is filtered through a micro aggregates filter and, then, re-infused, with or without previous treatments of the red blood cells. The blood obtained with this procedure may be diluted, haemolysed, defibrinated and might contains high concentration of cytokines; for this reason, it has to be used in small amounts and within the first 6 hours after the collection.

Despite the intracavitary blood usually does not clot because it defribrinates when enters in contact with a serous membrane, this process takes about one hour to be complete (Purvis, 1995). If the bleeding occurs very quickly, or if big vessels are damaged, clots could also be collected. For this reason, is always a good practice to add anticoagulants to the blood, in a ratio at least of 1:7, and to use micro filter, in order to catch even the smallest clots.

In general, autologous blood transfusion has been described to be a good cost-effective treatment in patients with acute blood losses induced, for example, by trauma or surgery. Advantages of autologous blood transfusion are the reduced incidence of adverse effects due to homologous blood transfusion, no transmission of infectious diseases, no need to determine the blood group and ready availability of blood when blood products are not easily available or affordable. Autologous blood transfusion should not be performed in human patients older than 80 years, weighing less than 50 kg, with cardiac diseases or other conditions such as Cushing disease, hypothyroidism, respiratory distress, cerebral stroke, hyperthermia, severe neutropoenia or thrombocytopenia, or congenital abnormalities of the red blood cells. Moreover, autotransfusion cannot be performed if the surgical area is contaminated with urine, faeces, fat or purulent material and is strongly contraindicated in oncologic surgery.

No significant complications have been described in association with auttransfusion. In the study of Barriot et al. (1988), no adverse reactions occurred after autotransfusion neither

significant haemolysis, as confirmed by the normal levels of free haemoglobin (Hgb) and potassium (K^+). More recently, several other studies have been done in human medicine on the use of autotransfusion in patients with haemothorax, mainly of traumatic origin (Ma et al., 2016; Harrison et al., 2014; Salhanick et al., 2011; Kamiyoshiharaet al., 2008; Baldan et al., 2006; Mc Ghee et al., 1999). Based on these studies, clinical advantages of autotransfusion in patients with haemothorax are the absence of incompatibility reactions, reduced risk of hypocalcaemia, hyperkalemia and acidosis compared to stored blood, increased capacity to carry oxygen, no need to warm the blood and lower costs (Kamiyoshihara et al., 2008). Potential disadvantages are: risk of sepsis if the blood is contaminated or collected in a not sterile way, risk of embolism, DIC, haemolysis and that increased concentration of free haemoglobin could cause acute tubular necrosis (complication that has never been reported in the literature). Moreover, intracavitary blood is considerably deficient in clotting factors and fibrinogen, so requiring possible significant concomitant factors replacement (Salhanick et al., 2011).

In veterinary medicine, only a few studies have described the use of autologous blood transfusion. The first study was carried out in dogs with experimentally induced haemothorax, in order to evaluate the influence of autotransfusion on the patient clotting factors and platelets values (Napoli et al., 1987). Lower levels of platelets, fibrinogen, FDPs, clotting factors V, VII, X, XI and XII were observed, together with a significant increase in PT and aPTT in patients than had undergone an autotransfusion, though without any clinical significant complications. Moreover, clotting alternations have been found to quickly solve within the next 24 hours, whereas platelet abnormalities lasted for a longer period (Moore at al., 1980).

A few studies have been performed on autologous blood transfusion in dogs with iatrogenic (leakage from surgical arterial ligatures), traumatic or infectious (*Angiostrongylus vasorum*) haemoperitoneum (Robinson et al., 2016; Hirst and Adamantos, 2012). The techniques used

in the two studies were a two-syringes technique (Robinson et al., 2016) and a red blood cells salvage device (Hirst and Adamantos, 2012).

Although in human medicine, the autologous blood transfusion has been used since several years in patients with haemothorax as an emergency and life-saving procedure, only one retrospective study has been found in the veterinary literature in which, among 25 dogs that received an autologous blood transfusion, only in 6 cases the autotransfusion was performed from haemothorax (Higgs et al., 2015).

This report describes a case of a haemothorax secondary to a coagulopathy disorder in a puppy, treated with the autologous blood transfusion.

2.1.1 Case presentation

This report describes the case of a 6-month old, mix-breed, male dog, of 12 kg body weight, presented to the Veterinary Teaching Hospital of the University of Messina because of lethargy and anorexia since one day. The dog lived free in a big garden where the owners were used to regularly place rodenticides in order to solve the problem of rats. Moreover, according to the owner, the dog was used to hunt rats. The night before the admission to the Emergency Department, the dog had not come back home and was found, the same morning, lying down in the garden. A few days before, the mother and the brother that lived in the same place, presented the same clinical signs and were diagnosed with dicumarine rodenticides intoxication by another veterinarian.

On general physical examination on admission, the dog was showing signs compatible with severe hypovolemic/haemorrhagic shock. The sensorium was severely lethargic, he was tachycardic (heart rate, HR, 205 beats/minute, bpm) with a weak peripheral pulse, his mucous membranes were light pink (Capillary Refill Time, CRT, > 2 seconds), he was tachypnoeic (respiratory rate, RR, 44 breaths/minute), and severely hypothermic (rectal temperature, T, of 28.7 °C). On chest auscultation and abdominal palpation no major abnormalities were found.

Immediately, oxygen supply though a *flow by* and active heating with UV lamp and isothermal blanket were started. Rectal temperature was constantly monitored through a rectal probe connected to a monitor (Patient care monitor HB100, Masimo SET, Hackermann&Bilt). A venous catheter was place on the cephalic vein and a bolus of 20 mL/kg of preheated Ringer Lactate Solution (Ringer Lattato S.A.L.F.,S.A.L.F. S.p.A. Laboratorio Farmacologico, Cenate Sotto, BG, Italy) was administered. When the venous catheter was placed, an haematoma and a loss of blood from the catheterisation site occurred. A microhaematocrit and the evaluation of Total Solid (TS), by mean of a refractometer (Giorgio Bormac s.r.l.), blood glucose, by mean of a portable glucose meter (Glucocard, Menarini, Firenze, Italy), and lactate, by mean of a portable lactate meter (Lactate Scout+, EKF Diagnostics), were performed from a drop of whole blood. These evaluations showed a severe anaemia (Packed Cell Volume, PCV: 10.5%; laboratory range: 37.3-61.7%), low total solids (TS 3.5 g/dL; laboratory range: 6.5-7.5 g/dL), hyperglycaemia (19.7 mmol/L; laboratory range: 3.9-6.1 mmol/L) and very high lactate (13.3 mmol/L; laboratory range: 0.5-2.5 mmol/L). Considering the severe anaemia and very low total solids, an acute haemorrhage was diagnosed. The owners were asked about blood losses during the previous days, but they did not notice anything, considering that the dog used to stay outside and had disappeared for the whole night. A sample of blood was taken from the lateral saphena vein; 1 mL was placed in a tube containing EDTA for the haematological profile, and 1 mL in a tube containing sodium citrate for the evaluation of clotting times. The haematological profile confirmed the severe anaemia (Red Blood Cell, RBC: 2.0 x 10^6/µL; laboratory range: 5.6-8.9 x 10^6/µL; haemoglobin, Hgb: 3.4 g/dL; laboratory range: 13.1-20.5 g/dL), leukocytosis (white blood cells, WBC: 30.0 x 10^3/µL; laboratory range: 5,05-16,76 x 10^3/µL) and slight thrombocytopenia (platelets, PLT: 84 x 10^3/µL, laboratory range: 148-464 x 10^3/µL). The evaluation of clotting times showed a severe prolongation of both PT (80 sec; laboratory range: 11-17 sec) and aPTT (150 sec; laboratory range: 72-102 sec).

Based on history, clinical signs and laboratory findings, a dicumarine rodenticides poisoning was the most likely diagnosis. Because of the presumptive diagnosis of rodenticides poisoning based on the anamnesis, clinical presentation and the result of the clotting times, it was decided to administer 20 mL/kg of fresh frozen plasma DEA 1.1 negative, at the rate of 0.5 mL/kg/h for the first 30 minutes, to eventually detect any anaphylactic reaction, and then it was gradually increased since the whole amount was administered in 6 hours. In the meantime, phytomenadione (Konakion®, Roche S.p.A., Segrate, MI, Italy) was started a dose of 5 mg/kg subcutaneously (SC) and, after the first dose, to 2.5 mg/kg orally (OS) two times per day (BID). The dog was recovered in an oxygen cage that provided up to 40% of oxygen for the first day, and then was moved to a normal cage for other 2 days. During the following days, the dog gradually improved and, after 4 days of hospitalization, he was alert, the pulse was strong (HR: 89 bpm) mucous membrane were pink (CRT <2 sec), RR was 28 breaths/min and rectal temperature was 38.5° C. Moreover, haematological profile were improved and clotting times came back within laboratory range (Table 1) and the dog was discharged with the prescription to continue the phytomenadione for other 4 weeks.

Two weeks later the dog was again readmitted to the Emergency service because of lethargy and anorexia since the day before, and dyspnoea since the same morning. During that period, the dog had continued to live in the same garden and to hunt rats. Moreover, treatment with phytomenadione was not regularly administered and was interrupted after only one week.

On general physical examination the dog was again very lethargic, the pulse was very weak and the dog showed tachycardia (HR: 185 bpm), mucous membrane were white (CRT > 2 sec), he was tachypnoeic (RR: 70 breaths/min) with shallow breathing, and low rectal temperature (T 35.9 °C). On chest auscultation the heart and lungs sounds were considerably decreased. On abdominal palpation, the abdomen was not painful, however the undulation was positive. The dog was considered to be again in shock, and the same stabilisation procedures, as on the first admission, were applied in order to re-establish euvolemia

(reanimation fluid therapy, oxygen flow by and active heating). Haematological profile and clotting times evaluation showed again moderate to severe anaemia, leukocytosis, low total solids and prolonged clotting times (Table 1).

Because of the clinical presentation, a pleural effusion, most likely haemothorax based on history, clinical and laboratory findings, was suspected. Because the dog was very dyspnoeic, it was decide to perform a *thoracic focus assessment with sonography for trauma* (TFAST) with a multifrequency microconvex probe of 5 MHz (Esaote My Lab Vet). The TFAST showed a severe and diffuse pleural effusion, with an irregular echogenic area closed to the pericardium, compatible with a big blood clot. An echoguided thoracocentesis was performed with a 22 *Gauge* butterfly needle connected through a three-way stop cock to a 10 mL syringe, in order to perform a decompression of the thorax. The skin from the 6^{th} to the 9^{th} intercostals space was clipped and, after a surgical scrub, the needle was entered through the 7^{th} intercostals space, on the medium-ventral side. Whole blood that did not clot was aspirated, confirming the diagnosis of haemothorax.

It was asked to the owners to perform a second plasma or blood transfusion, but they declined because of financial restraint due to the cost of the previous hospitalization of the same dog. On the contrary, they agreed to perform an autologous blood transfusion.

Autotransfusion was immediately started, after the informed consent had been provided by the owner. The blood was aspirated in a sterile way with a new 22 *Gauge* butterfly needle connected, through a three-way stop cock to a 50 mL syringe containing the anticoagulant CPDA-1, in a ratio of 1:20 (at least 0.05 mL of anticoagulant for each mL of blood). At the end of the procedure, 80 mL in total of blood were collected (6.7 mL/kg) with a PCV of 27%. Immediately after the end of the thoracocentesis, the dyspnoea improved, but the RR was still high (60 breaths/min) and cardiovascular conditions were improved but still not stable. The blood collected was immediately administered through an infusion tube with a micro filter with pores of 200 µm. The venous catheter was previously flushed with NaCl 0.9% to remove

any trace of Ringer Lactate. Blood was administered at a rate of 20 mL/kg/h through a syringe pump.

Immediately after the end of the autotransfusion, the pulse became stronger (HR: 90 bpm), mucous membrane pinker (CRT <2 sec) and also the respiratory rate gradually decreased.

After the stabilization of the vital parameters, further diagnostic examination were performed. Abdominal ultrasound showed a mild abdominal effusion, while chest X-rays showed a bronco-interstitial pattern, compatible with bronchopneumonia or, most likely, with a pulmonary bleeding and haemorrhage, common in dogs with rodenticides intoxication (Sheafor and Couto, 1999), and a residual slight amount of pleural effusion.

After the stabilization and the additional diagnostics, the dog was hospitalized in the oxygen cage for 3 days. Maintenance fluid therapy with Ringer Lactate was started and phytomenadione was re-started at the loading dose of 5 mg/kg SC and then switched to 2.5 mg/kg OS. Because of the radiological findings and of the leukocytosis, amoxicillin and clavulanic acid (Amoxicillina E Acido Clavulanico Teva 1000 mg, Assago, Italy) 20 mg/kg IV BID was started. A vitamin B complex (Stimulfos®, Teknofarma SpA, Torino, TO, Italy) was added to maintenance fluid therapy.

After 10 days of hospitalization, the dog was clinically stable (alert, pink mucous membranes, CRT< 2 sec; HR: 90 bpm with a strong pulse; RR: 18 breaths/min; rectal temperature: 38 °C), haematological profile and clotting times were within the laboratory ranges, and the dog was discharged with the prescription to continue the phytomenadione for other 3 weeks and to perform the *coupage* at least two times per day.

On follow-up after 15 days, the dog was clinically stable and was completely normal on chest auscultation.

One year later, the dog was readmitted to the Emergency Service because of the sudden onset of seizure, but despite the treatment and the sedation, the epileptic status never disappeared completely and the dog died 3 days after. Toxicological examination performed by the Istituto

Zooprofilattico Sperimentale (IZS) of Sicily “A. Mirri” confirmed an organochlorine compounds intoxication, as already diagnosed in other dogs living in the same area of the city.

Table 1. Laboratory findings (blood glucose and lactate, total solids [TS], prothrombin time [PT], activated partial thromboplastin time [aPTT], packed cell volume [PCV], haemoglobin [Hgb], red blood cells [RBC], white blood cells [WBC] and platelets [PLT]) on admission and discharge from the hospital on the first and second hospitalization

Parameter	First hospitalization		Second hospitalization	
	Admission	*Discharge*	*Admission*	*Discharge*
Blood glucose *(3,9-6,1mmol/L)*	19.67	6.5	3,72	5,8
Blood lactate *(0,5-2,5 mmol/L)*	13,3	2,0	7,8	2,1
TS *(6.0-8.5 g/dL)*	3.5	6.3	3.7	6.5
PT *(11-17 sec)*	80	12	30	10
aPTT *(72-102 sec)*	150	82	121	93
PCV *(30-45 %)*	10,5	25,3	19,5	31,6
Hgb *(13.0-18.0 g/dL)*	3.4	8.3	6.5	10.9
RBC *(5,6-8,9 M/µL)*	2,00	4,2	3,3	5,7
WBC *(5,1- 16,8 K/µL)*	30,0	11,8	51,5	25,4
PLT *(148-484 K/µL)*	84	123	267	242

2.1.2 Discussion

The present report describes a case of coagulopathy haemothorax in a puppy exposed to dicumarine rodenticides. On the first admission, the history (exposition to dicumarine rodenticides), clinical and laboratory findings (severe haemorrhagic anaemia and severe prolongation of the PT together with only a slight prolongation of the aPTT) confirmed a secondary haemostasis disorder and strongly lead to the suspicion of dicumarine rodenticides intoxication. In fact, among the other causes of a secondary haemostasis disorder, haemophilias A and B should have caused an increase only of the aPTT level, while a liver disease should have cause equal alterations of both the PT and the aPTT. In the case reported in this study, the PT was more than 7 and 2 times higher than the upper laboratory range on the first and the second admission, respectively, while the aPTT, on both admissions, was only slightly prolonged. Among the other differential diagnosis, a Disseminated Intravascular Coagulation (DIC) could not be ruled out, considering also the mild thrombocytopenia, but it seemed unlikely considering the history and the prompt response to the treatment. In fact, although the transfusion of fresh frozen plasma performed during the first hospitalization, could have been the cause of the normalization of clotting times, during the second hospitalization phytomenadione was administered as the only medical treatment. Deficit of the primary haemostasis, like von Willebrand disease (vWD), seemed, as well, unlikely because they rarely cause such severe alterations of PT and aPTT values. Moreover, after the second hospitalization, the dog had never showed again any other bleeding problem.

The autologous blood transfusion administered in this case, resulted to be an effective, easy to perform and less expensive treatment, compared with conventional homologous blood transfusion, able to solve the haemorrhagic shock and to improve the anaemia. As showed in this report, autotransfusion could be a good alternative treatment in acute haemorrhage when blood products are not easily and quickly available or when, like in this case, financial concerns exist. In the case described in this report, on the second admission to the Emergency

service, the owner showed concerns about the cost of a second blood or plasma transfusion. For these reasons, it was decided to perform an autologous blood transfusion with the blood collected from the thorax.

Blood collection was performed through a syringe technique, as already reported in the other cases of autotransfusion in patients with haemothorax (Higgs et al., 2015). Although free blood that is in contact with pleural or peritoneal surfaces for more than 1 hour becomes defibrinated and does not clot (Purvis, 1995) and the use of an anticoagulant may not be required, in this case it was decided to add CPDA-1 to the collected blood because an acute traumatic cause, with damage of big vessels, could not be excluded on initial presentation. Although massive transfusions with blood product containing ACD anticoagulant have been reported to cause hypocalcaemia, most likely resulting from the citrate binding the circulating calcium and magnesium, thus decreasing their serum concentrations (Jutkowitz et al., 2002), in the case described in this report, the amount of blood transfused was not massive (6.7 mL/kg), and no signs related to hypocalcaemia (muscle tremors, hypotension or arrhythmias) have occurred during the whole hospitalization period after autotransfusion. Furthermore, considering the suspect of dicumarine intoxication, the amount of anticoagulant used was the lowest possible (1:20 ratio). In the veterinary literature it has been recommended to add either 0.05–0.07 mL of anticoagulant per milliliter of collected blood (Purvis, 1995) or 0.14 mL of anticoagulant per milliliter of collected blood. (Jutkowitz, 2004). Moreover, in the study of Higgs et al., (2015), hypocalcaemia was detected in the 25% of dogs tested, and none of them showed related clinical signs. However, if multiple plasma and blood transfusions are required in addition to the autologous blood transfusion, in order to stabilize a severe coagulopathy disorder, the type and whole amount of anticoagulant used should be taken into consideration and calcium and magnesium blood levels should be monitored.

Other techniques for homologous blood transfusion have been reported in dogs. Among them, the red blood cells salvage device has been used in dogs with haemoperitoneum (Kellett-

Gregory et al., 2013; Hirst and Adamantos, 2012). Blood, collected from the abdominal cavity during surgery or from post-surgical drains, was washed with NaCl 0.9% in order to remove plasma proteins, cellular components, free haemoglobin, bacteria and activators of coagulation and inflammation, minimizing the risk of adverse reactions. Despite the potential benefits of this technique compared to the others used for autotransfusion, the two main limitations are the need of a big amount of blood and the cost of the device. Moreover, a significant higher risk of developing hypocalcaemia and hypomagnesaemia was described in association with this technique (Lamb et al., 2015). However, information on complications, contraindications and risks associated with cell salvage are still lacking in the veterinary literature, and further investigations are warranted.

In the case described in this report, considering the ongoing hypovolemic shock, the blood was administered at a high rate of infusion (20 mL/kg/h) without any complications, as reported in previous studies (Robinson et a., 2016). Considering that the blood was collected in a 50 mL syringe, it was decided to use a syringe pump for the administration, in order to reduce as much as possible blood manipulations. Although it has been reported in dogs that RBCs administered via syringe pumps have a marked decreased half life compared to delivered by gravity flow RBCs (McDevitt et al., 2011), in this case the autotransfusion was aimed at solving the acute hemorrhagic shock.

Advantages of autotransfusion compared to homologous blood, reported in the literature are: no transmission of infectious disease, no risk of immunization to blood and protein antigens and anaphylactic reactions, ready availability of fresh blood without the need to perform blood typing and cross-matching, prevention of hypothermia because the blood has almost the same temperature of the body, without no need of warming, and higher level of 2,3-diphosphoglycerate (2,3 DPG), which shifts the oxygen dissociation curve to the left, resulting in increased affinity of haemoglobin for oxygen and increased delivery of oxygen to the tissues (Robinson et al., 2016).

Complications reported in association to autotransfusion are haemolysis, coagulation disorders, microembolism of fat or air, microaggregate of platelets, and sepsis. Moreover, the contact with air should be avoided as it can lead to platelet aggregation and subsequent thrombocytopenia as well as haemolysis (Robinson et al., 2016). Consumptive and dilution coagulopathy have been reported with autologous blood transfusion, with an increase in clotting times that, anyhow, usually self-correct within the first 72 hours after transfusion without determining significant clinical signs (Pruvis, 1995). Moreover, this changes could happen also together with homologous blood transfusion. Moreover, in stored blood platelets death occurs within 2 days of storage, and this could lead to patient dilution thrombocytopenia. Therefore, using auto-transfused blood should help preventing the depletion of platelet numbers when compared to stored blood products (Robinson et a., 2016). Other problems related to the administration of stored blood are increased serum concentrations of total iron, haemoglobin and ferritin, as a result of intravascular haemolysis (Wurlod et al., 2015) and the induction of a pro-inflammatory cytokine response (Callan et al., 2013), compared to fresh blood. High levels of free haemoglobin have been associated with kidney damage in the dog (Harrison et al., 1947). However, this complication has never been reported in association with autotransfusion.

Survival after autologous blood transfusion has been reported to be 68%, and the cause of death was not related to autotransfusion complications (Higgs et al., 2015).

Although several study have been carried out in human medicine on the use of autologous blood transfusion in patients with haemothorax, only a few reports on this topic exist in the veterinary literature (Napoli et al., 1987; Higgs et al., 2015). Moreover, only one retrospective study exists which describes the use of this technique in dogs with haemothorax (Higgs et al., 2015). The present report would be a contribution to the limited knowledge of autologous blood transfusion in dogs with haemothorax. This report shows that this technique could be a good alternative to homologous blood transfusion in emergency situation, when blood

products are not available or indicated. The technique used was easy to perform, without the need of specific and expensive equipments. However, in case of severe coagulopathy disorders, thoracocentesis, as well as abdominocentesis and pericardiocentesis, should be performed when the patient's clinical condition is extremely severe (severe dyspnoea, life-threatening anaemia with no blood products available) and the procedure itself could be life saving, because, in these patients, even a small needle could cause a further worsening of the bleeding.

In conclusion, although autotransfusion is not a causal treatment, it seems to be an effective technique for the stabilization of emergency patients with acute haemorrhage. Further studies should be carried out in order to define a standardization of the technique and to provide more information about eventual complications and contraindications.

2.2 Acute Hypoxaemic Respiratory Failure With Haemoptysis in a Dog Exposed to Copper Sulphate Powder

Copper is an essential trace element involved in complex enzymatic reactions. Both deficiency and excessive intake can lead to life-threatening complications (Fieten et al., 2012). Its high redox activity can be harmful to cells by inducing oxidative stress, which will finally lead to cell death (Bulcke et al., 2015). Copper sulphate ($CuSO_4$) is commercially available as powder or water solution and it is widely used as pesticide.

Acute and chronic copper poisoning is well described in food animals (Christodoulopoulos and Roubies, 2007; Cornish et al., 2007; Ortolani et L., 2004). In humans, it is more often chronic and accidental (occupation-related) but some voluntary poisonings have been described as a uncommon attempt of suicide (Gamakaranage et al., 2011; Franchitto et al., 2008) or abortion (Motlhatlhedia et al., 2014). In companion animals there is a lack of information about copper toxicity. The only reference concerns to the use of copper sulphate to induce emesis (Percie du Sert et al., 2012).

Although is well known that copper sulphate could induce mucosal lesions after ingestion, studies concerning its respiratory toxicity lack, both in human and veterinary literature. The so-called “Vineyard sprayer's lung” (Eckert and Jerochin, 1982) and several adverse effects after exposition and inhalation of copper fumes in humans have been reported (Armstrong et al., 1983). *In vitro* studies have demonstrated that copper-sulphides and copper oxide nanoparticles (CuO NPs) induce cytotoxicity and oxidative stress in cultivated human lung cells, up-regulating cellular reactive oxygen species (ROS) and causing cell death (Harrington et al., 2015; Jing et al., 2015). Antioxidant N-acetylcysteine reduced CuONPs, which cause cytotoxicity, by lowering the production of intracellular ROS (Jing et al., 2015). Some authors have shown that intra-tracheal instillation of CuO NPs induced oxidative stress, inflammation and neoplastic lesions in rats (Ahamed et al., 2015).

This report describes a case of cupric poisoning with acute respiratory failure in a young dog after exposure to a copper sulphate powder, used as anticryptogamic.

2.2.1 Case presentation

A 2-year old male mongrel dog was presented to the Veterinary Teaching Hospital of the University of Messina because of the onset of a dry cough. About sixteen hours before, the dog was exposed to a copper sulphate pesticide (pentahydrate copper sulphate >98-99%; Solfato di rame, Manica S.p.a., Rovereto, TN, Italy) that the owner was spraying in the vineyard. The owner saw the dog sniffing in the bag of copper sulphate powder and, after that, he noticed blue powder spots on its nose. Until that moment, the dog had always enjoyed good health.

During the first physical examination, the dog was alert and no clinical abnormalities were found, except for a dry cough easily elicited on tracheal palpation. No alterations were found on chest auscultation. Haematological and serum biochemistry profiles, as well as urinalysis, were unremarkable.

Based on the effects of copper sulphate reported in literature, possible gastrointestinal and/or haematological involvements were suspected. Therefore, the dog was discharged with gastro-protective, detoxifying and antioxidant preventive therapy.

About three hours later, the dog was readmitted to the hospital because the owner noticed a small blood spot on the floor and because of a gradual loss of liveliness.

The dog was alert but lethargic; at physical examination, tachycardia (heart rate, HR: 112 beats/minute, bpm) and tachypnoea (respiratory rate, RR:36 breaths/minute), prolonged capillary refill time (CRT) of 3 seconds, pale mucous membranes, weak and fast pulse, and normal rectal temperature were found. Auscultation of the lungs was unremarkable.

Microhaematocrit was slightly low (PCV: 33%; laboratory range: 37-55%), total solids, evaluated by mean of a refractometer, were within the physiological values (TS: 7 g/dL;

laboratory range: 6.5-7.5 g/dL), while the lactate, measured by means of a portable lactacidometer (Lactate Scout +, EKF Diagnostics), was slightly high (3.8 mmol/L; normal value: <2.5mmol/L), suggesting inadequate tissue perfusion.

Fluid therapy with lactate Ringer's solution (Ringer lactate solution S.A.L.F. S.p.A., Cenate Sotto, BG, Italy) was started, at a rate of 10 mL/kg/h. After 15-minutes of infusion, an improvement in perfusion, with normalization of CRT and reduction of the lactate (3.2 mmol/L), was observed. In contrast, the breathing became faster (RR: 52 breaths/min) and shallow, cough worsened and haemoptysis occurred. Chest auscultation revealed bilateral end-inspiratory crackles.

Fluid therapy was immediately stopped, supplemental oxygen (flow-by) was provided and a chest X-ray (CXR) was taken. This showed a radiating peri-hilar interstitial pattern and patchy alveolar infiltrates, indicative of pulmonary oedema and/or haemorrhage, a luminal narrowing at the tracheal bifurcation, due to mediastinal lymphoadenopathy, and a vascular pattern due to vascular congestion. Adjacent lung fields appeared normal (Figure 1).

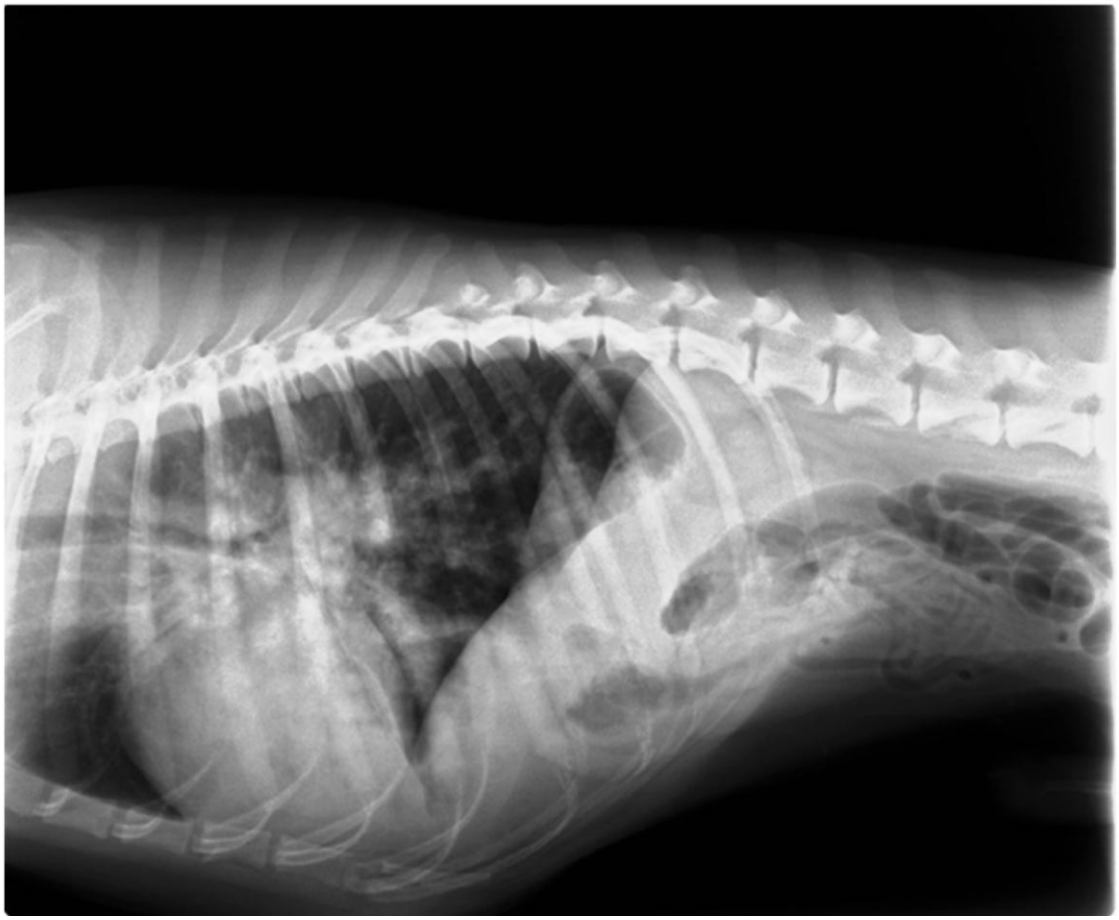


Figure 1. Day 1 (afternoon). Chest X-Ray: radiating peri-hilar interstitial pattern and patchy alveolar infiltrates; luminal narrowing at the tracheal bifurcation and vascular congestion

Arterial blood gas (ABG) analysis (Vet Scan®, i-STAT Analyzer Abaxis) showed a mixed disorder characterized by normal pH, as the effect of slight metabolic acidosis and respiratory alkalosis, with hypocapnia and severe hypoxia (Table 1).

The haematological profile showed normocytic normochromic anaemia, leukocytosis, with neutrophilia, monocytosis and eosinopenia, and thrombocytopenia (Table 1). A blood sample was collected into tubes containing sodium citrate in order to evaluate clotting times.

Clinical examination and radiological, haematological and blood gas findings supported the diagnosis of an acute hypoxemic respiratory failure (AHRF) probably due to pulmonary oedema and haemorrhage. The aetiological hypothesis considered in the differential diagnosis were: aspiration pneumonia, pulmonary contusion, inhalation of hot or toxic gases, drug or toxin exposures (i.e. anticoagulant rodenticide poisoning) and other causes of acute respiratory distress syndrome (ARDS) (Kelmer et al., 2012).

In order to reduce respiratory distress, oedema and pulmonary haemorrhage, methadone (Semfortan®, Eurovet Animal Health B.V., Sagrate, MI, Italy) 0.1 mg/kg intramuscularly, IM; every 6 hours, furosemide (Diuren®, Teknofarma S.p.a., Turin, Italy) 2 mg/kg intravenously, IV; twice a day, BID and tranexamic acid (Ugurol®, Rottapharm S.p.a., Monza e Brianza, Italy) 15 mg/Kg IV, BID, were administered respectively.

The dog was hospitalized in an oxygen cage. The respiratory rate was constantly monitored and methadone was repeated every 6 hours.

To the initial therapy, the following drugs were added: ceftriaxone (Ceftriaxone Hexal S.p.a., Agrate Brianza, Monza e Brianza, Italy) 50 mg/kg IV; once a day, SID and doxycycline (Ronaxan®, Merial Italia S.p.a., Noventa Padovana, PD, Italy) 10 mg/kg orally, OS; SID, to control a probable bacterial pneumonia; N-acetylcysteine (Fluimucil®, Zambon Italia s.r.l., Bresso, MI, Italy) 140 mg/Kg in bolus followed by 70 mg/kg IV, SID, and glutathione (TAD®, Biomedica Foscama Industria Chimico-Farmaceutica S.p.a., Ferentino, FR, Italy) 33 mg/kg IV, SID, for their antioxidant effects; cyanocobalamin/thiamine association (Dobetin

B1® 10,000, Esteve S.p.a., Milan, Italy) IV SID, as hepatoprotector/anti-anaemic support; metoclopramide (Vomend®, Eurovet Animal Health B.V., Bladel, The Netherland) 0.2 mg/kg IV BID, and ranitidine (Zantadine®, Ceva Salute Animale S.p.a., Agrate Brianza, Monza e Brianza, Italy) 1 mg/kg IV BID, as antiemetic and gastro-protective therapy; phytomenadione (Konakion®, Roche S.p.a., Monza e Brianza, Italy), 5 mg/kg IV BID, pending the results of coagulation tests, because the history could not exclude an anticoagulant rodenticide poisoning.

The ABG analysis performed after 6 hours showed a worsening in the results, with respiratory alkalosis, more severe hypoxia and metabolic acidosis due to hypoxic hyperlactataemia (Table 1). CXR confirmed the worsening with development into pneumonia (Figure 2).

The next morning, ABG and CXR were performed. The ABG showed a further increase in blood pH as the result of an improvement of both metabolic acidosis and respiratory alkalosis, likely due to the normalization of the respiratory rate. Despite the increase of the fraction of inspired oxygen (FiO_2), due to the oxygen-enriched air in the oxygen cage (about 40%), the arterial partial oxygen pressure (PaO_2) remained unchanged (Table 1). The CXR showed a worsening with a diffuse alveolar pattern in the cranial lobes (Figure 3). The haematological analysis showed an improvement of the anaemia but a worsening of the leukocytosis (Table 1). The coagulation results came out to be within the laboratory range, so coumarin poisoning could be excluded from the differential diagnosis and phytomenadione therapy was stopped.

In the following hours, a slight and gradual progress in respiratory signs was recorded and the CXR, carried out after about nine hours, showed an improvement in the interstitial pattern (Figure 4).

On the third day, the CXR showed a significant improvement in both the alveolar and the interstitial pattern, together with vascular decongestion (Figure 5). Methadone was reduced progressively over the next 24 hours.

On the fourth day, the CXR showed a further improvement (Figure 6). The ABG showed a slight alkalemia, as the effect of a mild respiratory and metabolic alkalosis probably due to the effect of furosemide. The hypoxia and the correlated lactacidemia also improved (Table 1). The haematological analysis showed a further increase in the number of erythrocytes and persistence of thrombocytopenia; the leukogram showed a decrease in neutrophils and an increase in monocytes, with normalization of eosinophil values (Table 1).

Methadone was withdrawn and the supplemental oxygen was progressively reduced until discharge within 6 hours.

An antibiotic therapy with enrofloxacin (Baytril®, Bayer S.p.a., Milan, Italy) 5 mg/Kg SC, SID was started and the previous treatments were continued using a more compliant administration route. Ceftriaxone, metoclopramide, glutathione were prescribed for another 3 days; ranitidine and furosemide for 5 days; enrofloxacin, acetylcysteine and vitamin B complex for 2 week, doxycycline for 3 weeks. Coupage was recommended several times per day.

Finally, after 3 weeks of follow-up, both the clinical and the radiographic examination (Figure 7) did not reveal any sign of pulmonary abnormalities and laboratory exams findings were within the normal ranges.

After more than two years, the patient is still alive and in good health.

Table 1. Arterial blood gas analysis, lactate and haematological findings throughout hospitalization

Parameter	Normal values	Day 1	Day 1 (after 6 h)	Day 2	Day 4
pH	7.35-7.45	7.42	7.48	7.54	7.48
HCO_3^- (mmol/L)	22-27	18.7	14.7	21.4	27.5
$PaCO_2$ (mm Hg)	35-45	28.9	19.6	24.8	37.1
PaO_2 (mm Hg)	80-110	58	44	44	64
PaO_2/FiO_2 (mm Hg)	>300	276	≤209*	≤209*	304
Lactate (mmol/L)	<2.5	3.20	7.88	7.70	6.71
RBC ($x10^{12}$/L)	5.65-8.87	4.39	/	5.01	5.28
PCV (%)	37.3-61.7	29.4	29	33.5	35.1
Hemoglobin (g/L)	131-205	106	/	121	128
Reticulocites ($x10^9$/L)	10-110	96.1	/	94.7	204.3
WBC ($x10^9$/L)	5.05-6.76	28.16	/	31.48	32.16
Neutrophils ($x10^9$/L)	2.95-11.64	24.11	/	26.68	24.64
Lymphocytes ($x10^9$/L)	1.05-5.10	1.97	/	2.48	3.22
Monocytes ($x10^9$/L)	0.16-1.12	2.05	/	2.31	4.20
Eosinophilis ($x10^9$/L)	0.06-1.23	0.01	/	0.00	0.08
Basophils ($x10^9$/L)	0.00-0.10	0.02	/	0.01	0.02
Platelets ($x10^9$/L)	148-484	92	/	92	91

**In oxygen cage (oxygen>21%)*

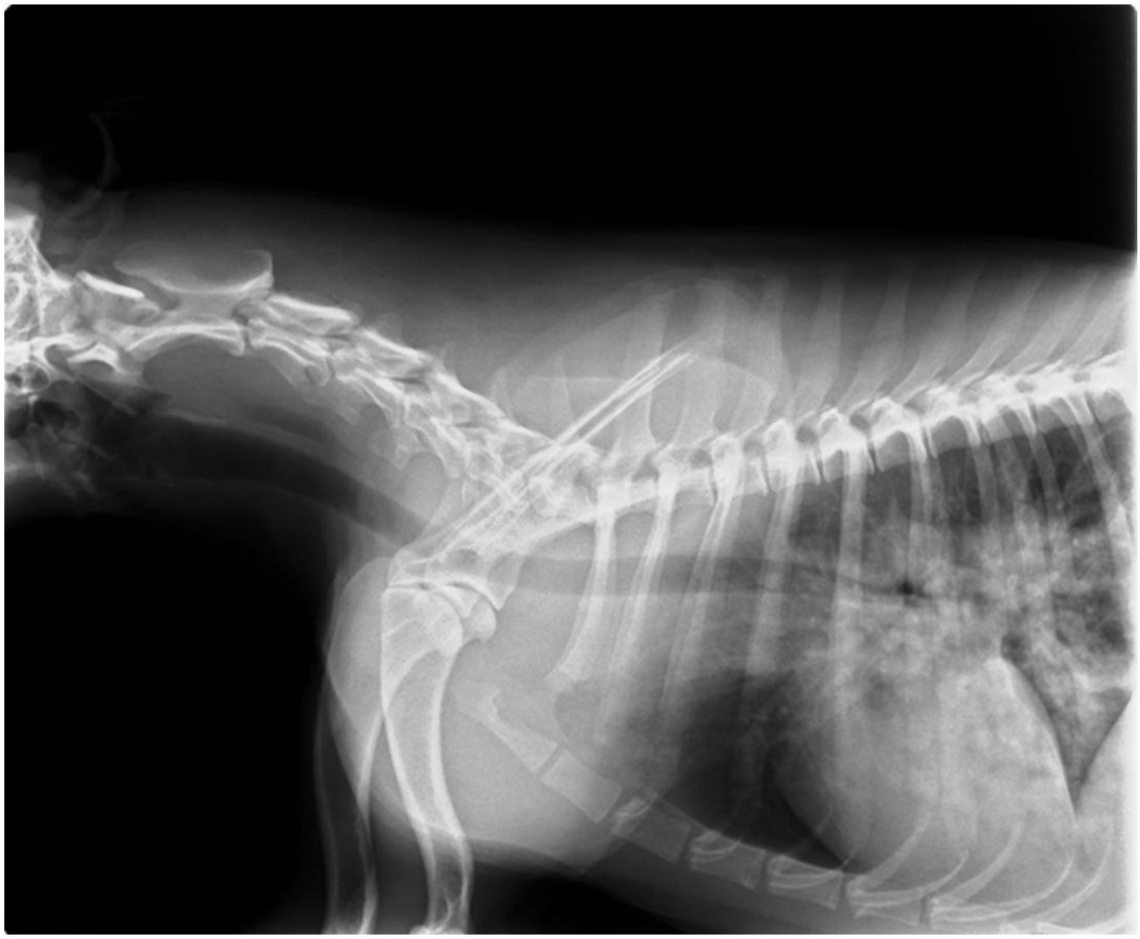


Figure 2. Day 1 (evening).CXR: worsening with development into pneumonia

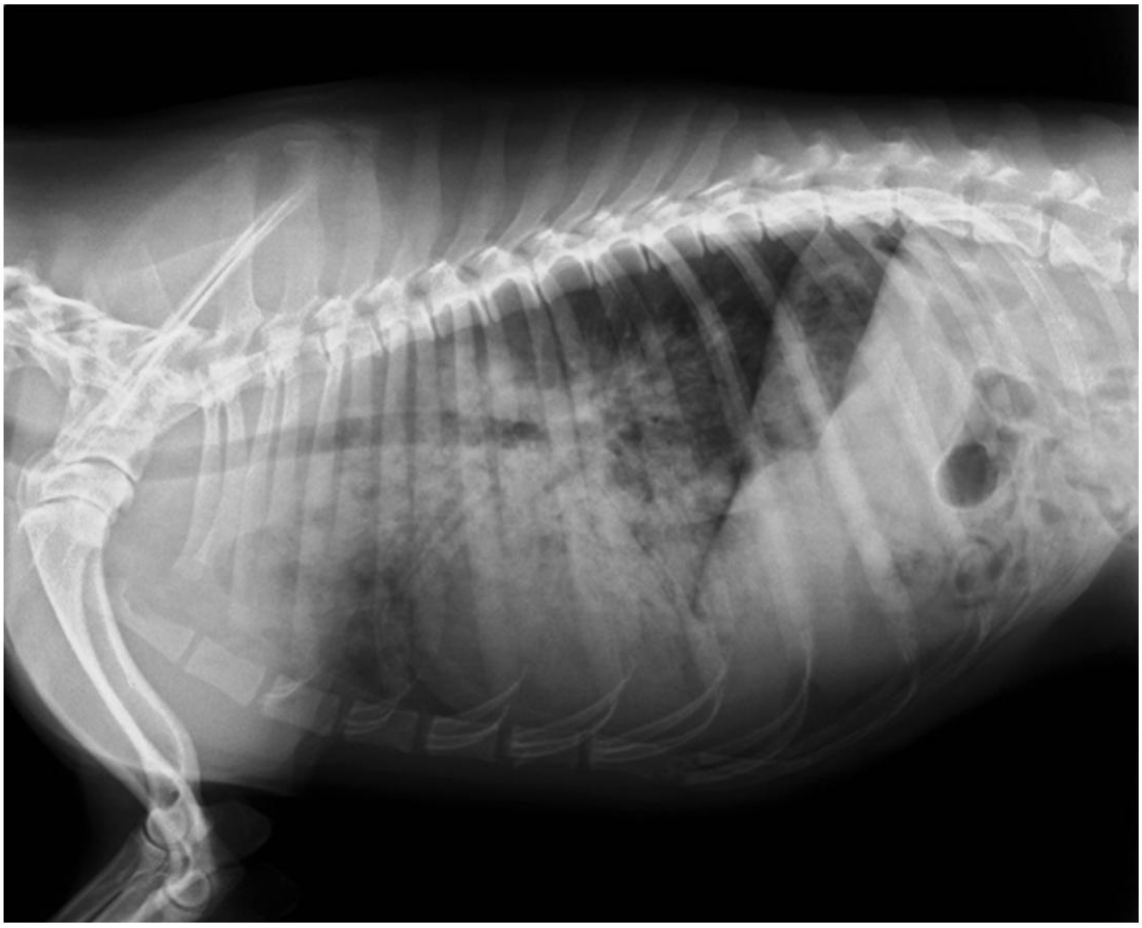


Figure 3. Day 2 (morning). CXR: further worsening with a diffuse alveolar pattern in the cranial lobes

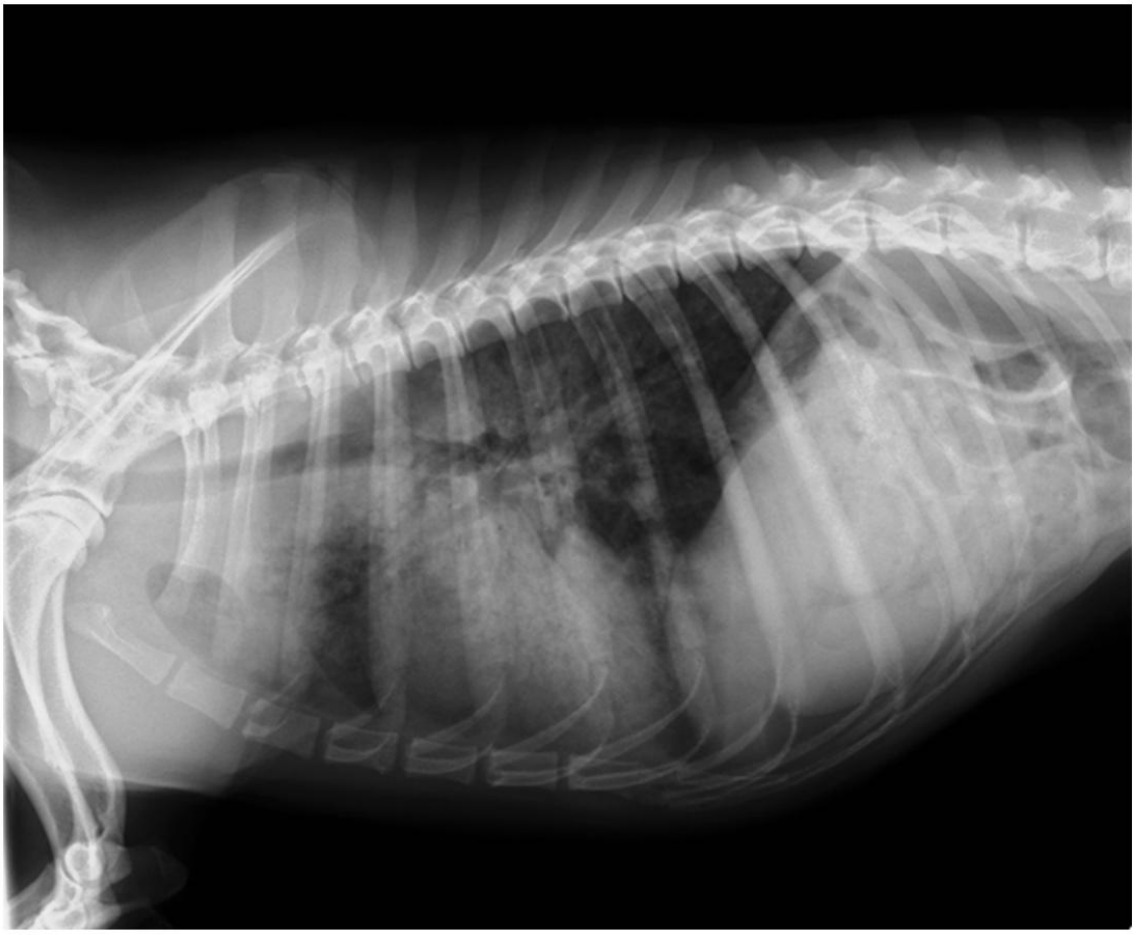


Figure 4. Day 2 (afternoon). CXR: improvement in the interstitial pattern

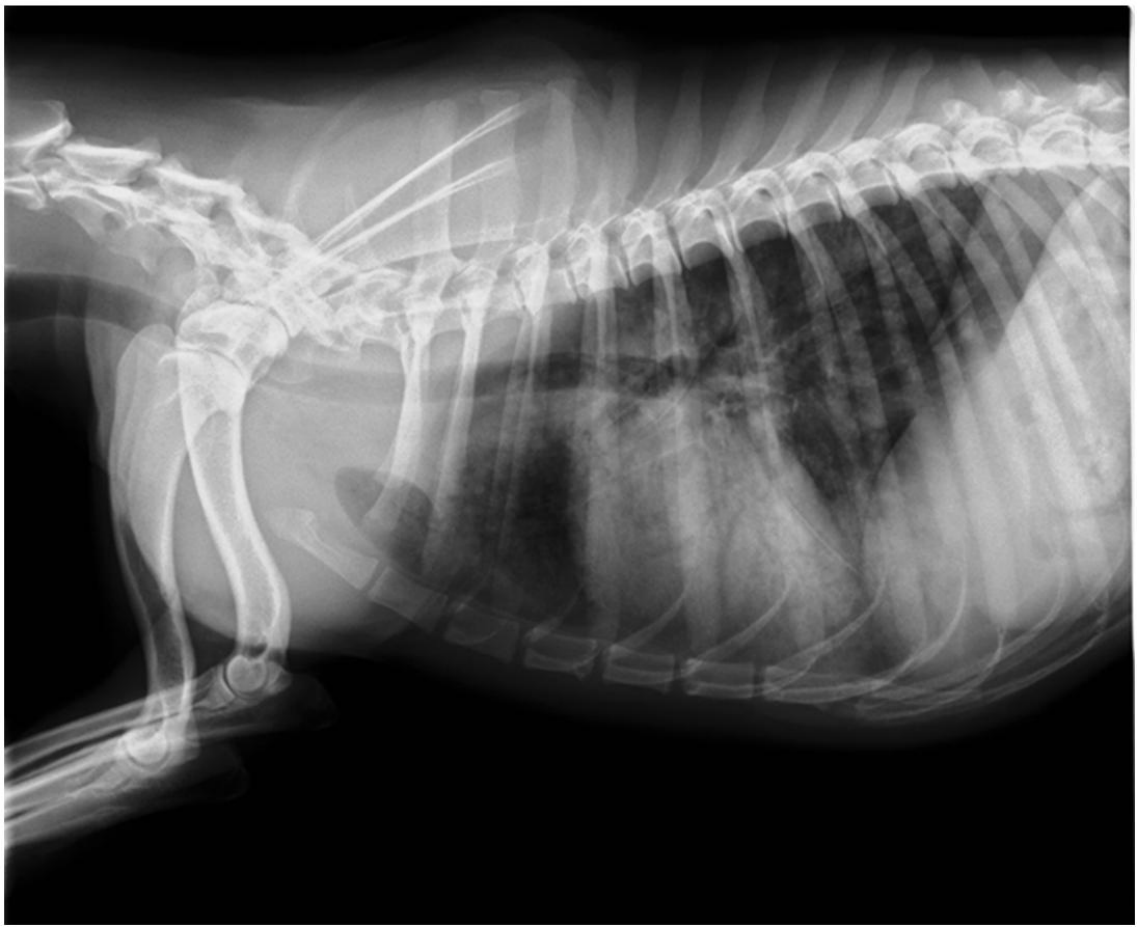


Figure 5. Day 3. CXR: significant improvement in both the alveolar and the interstitial pattern, and vascular decongestion

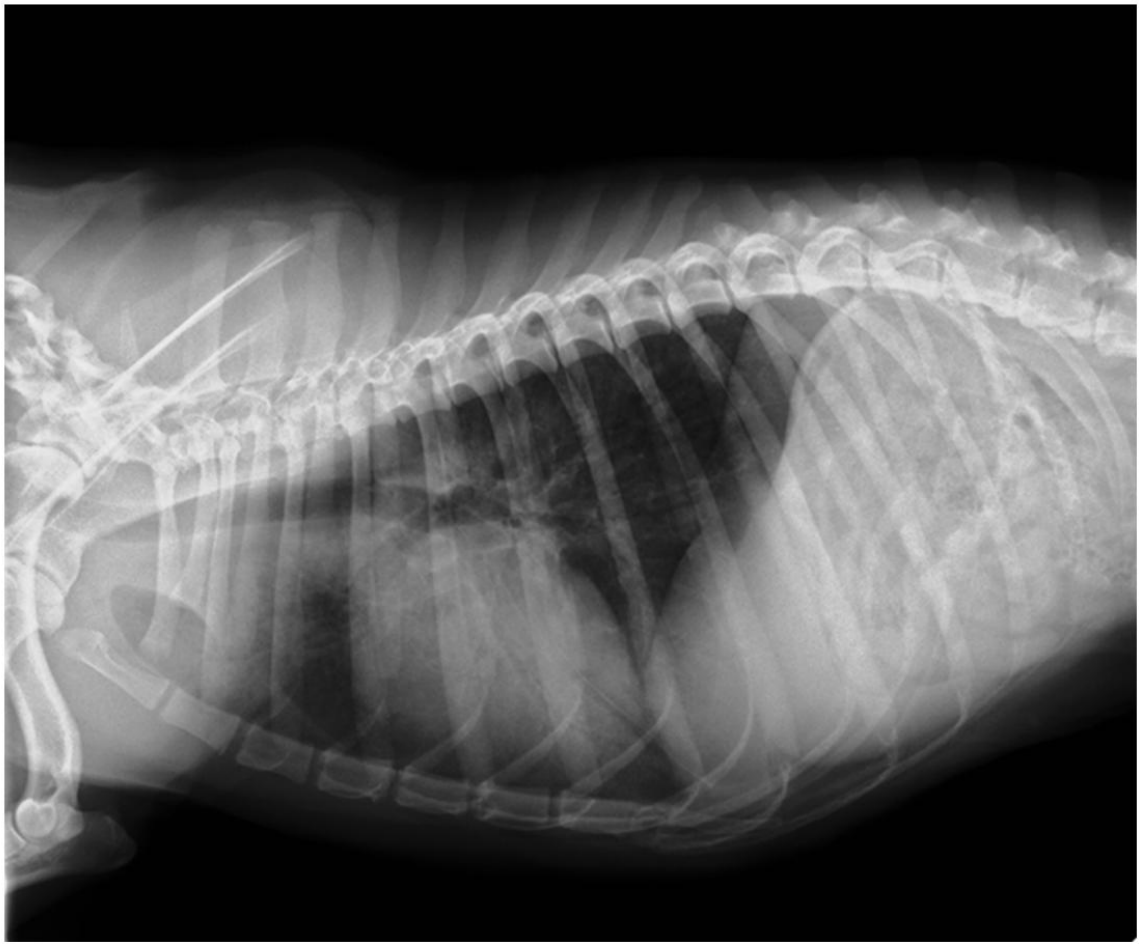


Figure 6. Day 4. CXR: further improvement; only a thin layer of diffuse interstitial pattern persisted

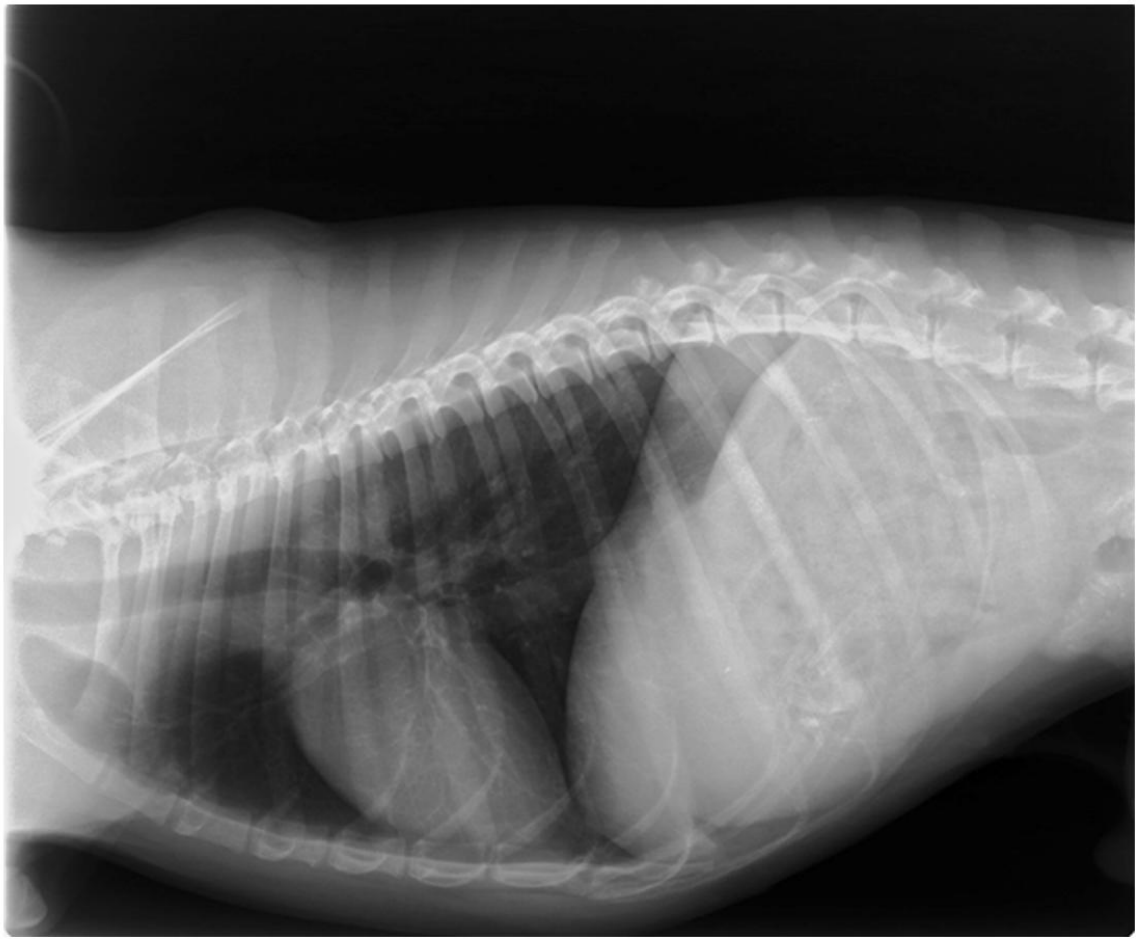


Figure 7. Day 21. Complete remission of CXR signs

2.2.2 Discussion

This case report was characterized by an acute respiratory failure with a rapid evolution, which made the prognosis guarded. At first, medical history and effects of copper sulphate reported in literature, had led towards the suspect of a possible gastrointestinal involvement but, thanks to ABG findings, it was possible to recognize a defect in gas exchange, as evidenced by the severe hypoxaemia refractory to supplemental O_2. Tachypnoea and compensatory hyperventilation resulted in hypocapnia with respiratory alkalosis.

Because of haemoptysis and regenerative normocytic normochromic anaemia, which arose within hours, we diagnosed a pulmonary haemorrhage, which fast led to pneumonia, as evidenced by the serial CXR findings and the developing of leukocytosis.

The initial PaO_2/FiO_2 ratio of 276 mmHg decreased despite oxygen supplementation. In humans, the Berlin criteria define mild (previously called Acute Lung Injury, ALI), moderate and severe ARDS when PaO_2/FiO_2 ratio is 200-300 mmHg, 100-200 mmHg and <100 mmHg, respectively (ARDS Definition Task Force, 2012). The mortality rates range between 40% and 60%, depending on the stage. In 2007, the "*Dorothy Russell Havemeyer Working Group on ALI and ARDS in Veterinary Medicine*" (Wilkins et al., 2007) established 5 criteria for the diagnosis of these conditions: 1) acute onset of dyspnoea from less than 72 hours; 2) recognizable risk factors, including direct and secondary pulmonary injury; 3) pulmonary capillary leak without evidence of a cardiogenic origin; 4) evidence of inefficient gas exchange; 5) evidence of diffuse pulmonary inflammation. The 5^{th} criterion, although strongly recommended, is optional due to the logistic constraints related to the performance of transtracheal wash or bronchoalveolar lavage (BAL) in a critically ill patient. Mortality rates have not been established in veterinary medicine. Survival from ALI/ARDS has been described in only few dogs in veterinary literature (Kelmer et al., 2012; Walker et al., 2005).

In this case, since in the oxygen cage the oxygen concentration is higher than atmospheric one, the PaO2/FiO2 ratio during hospitalization was probably <200 mmHg. At discharge, the ratio was still low, but a PaO_2 >60 mmHg provides satisfactory tissue O_2 delivery.

AHRF is caused by intrapulmonary shunting of blood resulting from airspace filling or collapse, which may result from: i) elevated alveolar capillary hydrostatic pressure, as occurs in left ventricular failure or hypervolemia; ii) increased alveolar permeability, as in any of the conditions predisposing to ARDS; iii) blood or inflammatory exudates.

The initial management of AHRF, regardless of the cause, is supporting vital functions. Hypoxaemia requires oxygen supplementation, but in the most complicated cases, mechanical ventilation may be required. Opioids are indicated to reduce respiratory distress. In patients with haemoptysis, tranexamic acid, an anti-fibrinolytic agent, may be a useful and well-tolerated medication. Current evidences indicate that this drug may reduce both the duration and the amount of bleeding, with a lower risk of short-term thromboembolic complications, even when used both through the bronchoscope and via inhalation (Moen et al., 2013; Solomonov et al., 2009).

The initial hypothesis of coumarin rodenticide poisoning was discarded due to the normality in the clotting times and the absence of other bleedings. Other causes of severe AHRF, such as sepsis, shock or cardiogenic pulmonary oedema were also excluded by clinical examination, as well as pulmonary contusion, inhalation of hot or toxic gases and drug exposures, based on dog history.

Hypervolemia was excluded, because total fluid volume given during the previous 24 hours was not excessive. However, the fluid therapy performed to treat the initial deficit of tissue perfusion, certainly contributed to the triggering and worsening of lung injury, presumably by increasing blood pressure in alveolar capillaries, already damaged.

Given the history and the distribution of pulmonary infiltrates, it has been hypothesized that the dog may have been an inhalation pneumonia. The hypothesis that is believed more likely

is that the dog inhaled an amount of copper sulphate powder enough to determine respiratory tree damage, extending from the trachea (initially) to the pulmonary alveoli. Because of the first haematic spot that the owner assumed to be vomit, it was not possible to exclude that a little amount of powder was also ingested, causing a slight gastric irritation, already described in humans (Gamakaranage et al., 2011; Franchitto et al., 2008). Therefore, an aspiration of gastric contents cannot be completely excluded. However, based on the description provided, it was more likely that even the hemorrhagic material seen by the owner came from the airways.

Considered the worsening in the ABG results after the first hours of hospitalization, the mechanical ventilation was strongly suggested, but the owner declined for economic reasons.

In literature, there are no evidences of copper sulphate poisoning in dogs and cats, unlike other species. The substance is harmful if swallowed and highly irritating after contact with skin and mucous membranes. The main toxic effects are expressed in the liver, blood and gastrointestinal tract. A strong irritation of the airways after inhalation has been described, although only a few specific reports exist. Inhalation of *Bordeaux Mixture* (1-2% $CuSO_4$) was reported to cause changes in the pulmonary interstitial tissues with respiratory insufficiency in vineyard workers. These lesions were reproduced experimentally in guinea-pigs and mices (Eckert and Jerochin, 1982). In addition, a case of fatal copper sulphate poisoning was described in a woman who died of inhalation pneumonia (Lamont and Duflou, 1988).

Clinical and experimental studies suggest that in the inhalation pneumonia one of the most important mediator is the oxidative stress induced by ROS (Guzel et al., 2008; Kanter et al., 2015), similarly to copper toxicity in tissues (Bulcke et al., 2015; Harrington et al., 2015; Jing et al., 2015; Ahamed et al., 2015). Therefore, it is possible that, even in the present report, this may have had an additive effect on lung cells. The administration of N-acetylcysteine and glutathione may have controlled the oxidative process by elevating glutathione peroxidase

levels, which are decreased in experimental models of aspiration pneumonia (Kanter et al., 2015), thus contributing to the good outcome of the dog.

No evidences exist on copper sulphate poisoning after inhalation, with consequent acute pulmonary involvement, in any species. This report would be a contribution to the current knowledge of copper poisoning, scarcely mentioned in literature both in human and veterinary medicine. Moreover, it has never been described in companion animals.

Finally, the severity of the clinical findings described in this report should be a warning for the careless use of potentially toxic substances in the presence of unattended pets, especially if they are young and naturally curious.

III. HAEMATOLOGY

3.1 Clinical efficacy of cyclosporine in aplastic pancytopenia associated with multiple CVBDs (Canine Vector-Borne Diseases) in a dog

Multiple infection by several vector-borne pathogens is common both in human and veterinary medicine. The pathogenesis and pathological consequences of concomitant infections are often not well understood. Several mechanisms related to transmission, host and cell invasion, immune response, pathogen multiplication and dissemination mechanisms, are probably involved. Co-infection can amplify pathogenic mechanisms and parasite transmission, making the progression of the disease worse and increasing its severity. Concomitant infection with several pathogenic agents may stimulate different host responses that could even allow the pathogens to act synergistically together with a more successful colonization of the human or veterinary host (Baneth, 2014).

Aplastic pancytopoenia, also called aplastic anaemia, is an uncommon disease of the dog. It is characterized by suppression of the bone marrow which ends up in progressive peripheral pancytopoenia (Milano and Dufour, 2015). In particular, the bone marrow of dogs with aplastic pancytopoenia appears hypocellular, with hematopoietic spaces replaced by adipose tissue (Brazzel and Weiss, 2006; Weiss et al., 1999). In dogs, only a few cases of aplastic pancytopoenia have been described. Among the described cases, the diagnosed or suspected causes of bone marrow hypoplasia were infectious diseases (parvovirosis, monocytic ehrlichiosis) (Brazzel and Weiss, 2006), drugs (estrogens, phenylbutazone, meclofenamic acid, cephalosporins, trimethoprim-sulfadiazine, albendazole, thiacetarsamide, captopril, griseofulvin, and quinidine) (Weiss and Klausner, 1990; Weiss and Adams, 1987), exogenous or endogenous estrogens toxicity (Sontas et al., 2009; Brazzel and Weiss, 2006; Suess et al.,

1992; Van Kruiningen and Friedland, 1987; Sherding et al., 1981), toxins, and exposure to radiation. There are also a few reports in which the potential cause was not detected, and the disease was considered idiopathic by exclusion (Kim et al., 2012; Brazzel et al., 2006; Weiss and Christopher, 1985). In humans, aplastic anaemia has been described also in association with viral hepatitis (Zhao et al., 2017).

Inclusion criteria for the diagnosis of aplastic pancytopoenia in humans are well defined and include hemoglobin concentration < 10 g/dL, low reticulocytes value, platelet count < 50 x 10^3/µL, neutrophil count < 1.5 x 10^3/µL, and hypocellular bone marrow on aspiration and core biopsy specimens. On the contrary, the inclusion criteria in veterinary medicine are still not well defined. Among them, the presence of pancytopoenia in the peripheral blood, concurrent evaluation of bone marrow aspirate and core biopsy specimens, and identification of a hypocellular marrow with >75% of the hematopoietic space replaced by adipose tissue, are generally considered indicative of the disease (Brazzel et al., 2006). However, the bone marrow cell distribution is not the same in all of its areas. For this reason, multiple samples would be ideal to achieve a certain diagnosis. However, most of the time, this is not feasible in dogs and cats because of the small body dimension and because the procedure is quite invasive and painful. Recently, magnetic resonance imaging (MRI) has been suggested as a valuable tool for non-invasive evaluation of bone marrow, due to its ability to differentiate hematopoietic and fatty marrow (Kim et al., 2012).

Therapeutic options in humans include supportive care and specific treatments (Milano and Dufour, 2015; Dolberg and Levy, 2014; Young et al., 2006). Supportive cares include administration of blood products and hematopoietic growth factors, and prevention/treatment of infections. Specific treatments include stem cell transplantation or immunosuppressive therapy, due to the proposed immune-mediated aetiology. Because bone marrow transplantation is currently not feasible in veterinary practice, due to the lack of compatible donors, immunosuppressive therapy is often the only specific treatment available. High doses

of corticosteroids may induce hematologic response in human patients, but chronic treatment may determine several and serious side effects. For this reason, combination of antithymocyte globulin and cyclosporine has been proposed as one of the most effective treatment (Shetty et al., 2016; Bacigalupo et al., 2000). Androgen therapy has been documented in veterinary (Eldor et al., 1978) and human medicine, but severe cases typically do not respond to androgen therapy alone. However, androgens can be considered as an adjunctive therapy to immune suppressors. In canine aplastic pancytopoenia, only a few trials of immunosuppressive therapy with high-dose prednisone or cyclosporine (CyA) have been reported with inconstant results (Kim et al., 2012; Barth et al., 1997). A good response to mycophenolate mofetil, an immunosuppressive drug used in human renal transplantation, has been reported in a dog in which combination therapy with prednisone, hematopoietic growth factors and cyclosporine has been ineffective (Yuki et al., 2007). Therefore, based on the available literature, therapeutic options for the treatment of aplastic pancytopoenia appear to be limited. Prognosis of aplastic pancytopoenia is generally considered poor (Brazzel and Weiss, 2006), and a relationship with the underlying cause has been found. In particular, dogs with pancytopoenia secondary to chemotherapy, ehrlichiosis, parvovirus infection, immune-mediated disease and sepsis have been found to be more likely to recovery from the disease; on the contrary, the prognosis was worse in dogs with estrogens toxicity, idiopathic aplastic anaemia, malignant histiocytosis and myelodysplastic syndrome (Weiss at al., 1999). However, even some dogs diagnosed with idiopathic aplastic pancytopoenia managed to recover after a long immune suppressive treatment (Kim et al., 2012; Yuki et al.., 2007).

This report describes the case of a dog with aplastic pancytopoenia associated to multiple CVBDs successfully treated with cyclosporine and aetiological therapies.

3.1.1 Case presentation

An adult (over 6-7 years old), intact female mongrel dog was presented to the Veterinary Teaching Hospital (VTH) of the University of Messina. She was a stray dog, found the day before in a rural area near Messina (Sicily, Italy).

On general physical examination, the dog showed lethargy, tremors and poor nutritional status (body condition score, BCS: 1.5/9; optimal score: 4-5/9), pale and sticky mucous membranes (capillary refill time, CRT, >2 seconds), weak pulse (heart rate, HR: 154 beats/minute, bpm), tachypnoea (respiratory rate, RR: 40 breaths/minute), fever (rectal temperature of 40 °C), 8% of dehydration and a massive infestation by ectoparasites. On palpation, all the lymph nodes were mildly enlarged. The skin was hypoelastic, with diffused seborrhoea and rarefaction of the coat, which appeared dry, opaque and with diffuse scurf. Moreover, she showed enlarged and swollen breasts, with colostrums secretion, and bloody vulvar discharge. For this reason, a recent pregnancy was likely. Appetite was good and general function were apparently normal. On abdominal palpation, an enlarged spleen was found. An abdominal ultrasound was performed and showed an enlarged spleen with normal echogenicity. No other abnormalities were found. Based on clinical findings, the dogs was considered to be in slight to mild shock. A venous catheter was placed on the cephalic vein, and a bolus of 20 mL/kg of Ringer Lactate Solution (Ringer Lattato S.A.L.F.,S.A.L.F. S.p.A. Laboratorio Farmacologico, Cenate Sotto, BG, Italy) was administered.

In the meantime, a blood sample was collected from the lateral saphena vein. The serum biochemistry profile showed only marked pre-renal uremia (BUN: 242 mg/dL; laboratory range: 20-45 mg/dL) and hyperproteinemia (total proteins 8.5 g/dL; laboratory range: 5.2-8.2 g/dL), while the complete cell blood count (CBC) profile showed severe normocytic, normochromic, non-regenerative anaemia (red blood cells, RBC: 1.48 x 10^6/µL, laboratory range:5.7-8.8 x 10^6/µL; haemoglobin, Hgb: 2.8 g/dL, laboratory range:12.9-18.4 g/dL; packed cell volume, PCV: 8.8%, laboratory range:37.1-57%; no reticulocytes, laboratory range: 3-50

x 10^3/µL), severe leucopoenia (white blood cells, WBC: 3.3 x 10^3/µL, laboratory range:5.2-13.9 x 10^3/µL) with neutropenia, and moderate thrombocytopenia (platelets, PLT: 68 x 10^3/µL, laboratory range:150-400 x 10^3/µL). Urinalysis, obtained through cystocentesis, was unremarkable. The blood group of the dog was tested and was DEA 1.1 negative. The thick drop test, examined at the optical microscope, showed several microfilariae (10 larvae in every low-power field, 10x). A modified Knott's concentration test was performed and microfilariae were morphologically identified as *Acanthocheilonema* (*Dipetalonema*) *reconditum* L_1 larvae. Based on clinical and laboratory findings, a multiple canine vector-borne disease (CVBD), largely diffused in the Mediterranean area (Pennisi et al., 2012; Baneth et al., 2008; Solano-Gallego et al., 2001), was suspected, and serum sample was sent to the Istituto Zooprofilattico Sperimentale (IZS) of Sicily "A. Mirri" for the research of antibodies (though immunofluorescent antibody test, IFAT) and DNA (through polymerase chain reaction, PCR) of the main vector-borne pathogens.

After the first bolus of Ringer Lactate, considering the severe anaemia, a fresh whole blood transfusion, collected from a DEA 1.1 negative donor, was started. Moreover, selamectin (Stronghold®, Pfizer Italia S.r.l, Italy) 10 mg/kg spot-on and a combination of fipronil/(S)-methoprene (Frontline Combo®, Merial Italia S.p.a., Italy) spray were administered to treat the massive infestation of ectoparasites.

While the results for the tick-borne pathogens were still pending, maintenance fluid therapy with Ringer Lactate and vitamin B complex (Stimulfos®, Teknofarma SpA, Torino, TO, Italy) 2 mL/10kg of body weight was continued, and imidocarb dipropionate (Carbesia®, MSD Animal Health S.r.l., Italy) 4 mg/kg intramuscularly (IM) and doxycycline (Ronaxan®, Merial Italia S.p.A., Italy) 10 mg/kg orally (OS) once per day (SID), were started.

After one week, imidocarb dipropionate administration was repeated, while the doxycycline was discontinued because of repeated vomiting and was replaced with enrofloxacin (Baytril®, Bayer S.p.a., Italy) 5 mg/Kg/day subcutaneously (SC). An anabolic steroid therapy with

stanozolol (Stargate®, Acme S.r.l., Italy) 5 mg/Kg IM once per week was started. The results of serological and molecular tests confirmed the CVBDs suspicion. Antibodies against *Ehrlichia canis, Anaplasma phagocytophilum, Rickettsia conorii, Babesia canis* and *Leishmania infantum*, and DNA of *E. canis* and *B. canis*, were detected.

During the following three weeks, despite stable clinical conditions and an improvement in body weight (+2 Kg), the CBC progressively worsened and there were no signs of improvement in haematopoiesis. In particular, besides the absence of reticulocytes, a significant leukopenia (WBC: 1.6 x 10^3/µL), with right shift of the Arneth index, and thrombocytopenia (PLT: 59 x 10^3/µL) were observed. A big number of microfilariae were still present into the peripheral blood at that time.

Due to the persistent peripheral pancytopenia, despite the aetiological treatment for tick-borne diseases (TBDs), bone marrow aspiration and core biopsy were performed and specimens were stained with May Grünwald-Giemsa and hematoxylin-eosin, respectively.

The bone marrow aspirate was poorly cellular and contained only few stromal cells, macrophages and fibroblasts. Hematopoietic precursor cells were not observed. Core biopsy showed a hypocellular marrow cavity filled with adipose tissue (>75%) and rare hematopoietic cells. Several extra- or intracellular (within macrophages) *Leishmania* spp. amastigotes were observed. Based on these findings, the dog was diagnosed with aplastic pancytopoenia and leishmaniasis. For this reason, an immune suppressive treatment with prednisolone (Vetsolone®, Bayer Spa Div. Sanità Animale, Milano, Italy) 2 mg/kg OS SID was started.

Because of leukopenia and fever (40.5°C), amoxicillin and clavulanic acid (Synulox®, Pfizer Pfizer Italia S.r.l, Italy) 12.5 mg/Kg OS two times per day (BID) was started. Because of the severe non-regenerative anaemia, another fresh whole blood transfusion was given 40 days after the first one.

During the following week, due to the occurrence of repeated febrile episodes (>40°C), the antibiotic association was changed with off-label ceftriaxone (Rocefin®, Roche S.p.A., Italy) 40 mg/Kg IV SID for 7 days, with clinical remission.

After other three weeks, although she continued to gain weight (7.5 Kg, BCS 5/9), the CBC showed a persistent peripheral pancytopenia (RBC: 3.08 x 10^6/µL; Hgb: 6.0 g/dL; PCV: 20%; no reticulocytes; WBC: 1.8 x 10^3/µL; PLT: 60 x 10^3/µL). In agreement with the owner, cyclosporine (Atoplus®, Novartis Farma S.p.A., Italy) 13 mg/kg OS SID was added to the immune suppressive therapy. Metronidazole (Flagyl®, Zambon Italia S.r.l, Italy) 25 mg/kg/day PO was added for its anti-*Leishmania* effects (Noli and Auxilia, 2005).

Already after one week, an improvement in the haematological profiles was found, and cyclosporine was reduced to 6.5 mg/kg/day divided in two doses. After the following two weeks, in addition to a further increase in body weight (8.3 Kg, BCS 5.5/9), the dog showed an improvement in CBC (RBC: 3.66 x 10^6/µL; Hb: 7.4 g/dL; PCV: 23.6%; WBC: 3.6 x 10^3/µL; PLT: 59 x 10^3/µL). Moreover, the anaemia started to be regenerative (reticulocytes: 1.46 x 10^3/µL). The thick drop test showed a reduction in the number of microfilariae.

Prednisolone was slowly reduced during the following month (1 mg/Kg/day PO for 10 days, 1 mg/Kg/day PO on alternate days for 10 days, and then 0.5 mg/Kg/day PO on alternate days for 10 days).

One month after the beginning of cyclosporine treatment, the CBC was further improved (RBC: 4.35 x 10^6/µL; Hb: 8.8 g/dL; PCV: 27.6%; WBC: 3.2 x 10^3/µL; PLT: 73 x 10^3/µL). However, during the following two weeks repeated febrile episodes (40-40.5°C), with anorexia, depression and tremors, occurred, and the CBC showed a rapid worsening (RBC: 3.54 x 10^6/µL; Hgb: 6.9 g/dL; PCV: 20.8%; WBC: 2.2 x 10^3/µL; PLT: 67 x 10^3/µL and, after another week, RBC: 2.64 x 10^6/µL; Hgb: 5.3 g/dL; PCV: 15.4%; WBC: 4 x 10^3/µL; PLT: 58 x 10^3/µL). A lymph node fine-needle aspiration (FNA) revealed a great number of *Leishmania* spp. amastigotes. Due to this findings, metronidazole was changed with

meglumine antimoniate (Glucantime®, Merial Italia S.p.a., Italy) 50 mg/kg SC BID for two months. Immediately after one day of treatment, a marked improvement in general condition and appetite was observed. During the following week, there was a gradual improvement in the CBC (RBC: 3.66 x 10^6/µL; Hb: 6.8 g/dL; PCV: 20%; WBC: 2 x 10^3/µL; PLT: 152 x 10^3/µL).

Eight weeks after the beginning of immune suppressive therapy, administration of cyclosporine was reduce to 6 mg/kg BID on alternate days, for other seven weeks. During this period, the CBC gradually improved and cyclosporine was stopped after 106 days of treatment. White blood cells, platelets and red blood cells came back within the laboratory ranges after the end of the treatment (RBC: 5.74 x 10^6/µL; Hgb: 14.7 g/dL; PCV: 31.8%; PLT: 191 x 10^3/µL; WBC: 3.5 x 10^3/µL). The thick drop test did not show any microfilaria.

The blood chemistry profile after the end of the treatment was unremarkable, except for a marked alteration of the protein pattern (total protein: 82 g/L). Serum proteins electrophoresis showed hypoalbuminemia (albumin: 13.6 g/L, laboratory range: 23-32 g/L), low α1 globulins (α1: 1.6 g/L, laboratory range: 2-5 g/L; α2: 4.8 g/L, laboratory range: 3-11 g/L), normal β globulins (13.4 g/L, laboratory range: 6-22 g/L) and severe hypergammaglobulinemia (γ globulins: 48.6 g/L, laboratory range: 9-22 g/L) with low albumin/globulins ratio (0.20, laboratory range: 0.8-1.7).

Based on this findings, allopurinol (Zyloric®, Teofarma S.r.l., Italy) 30 mg/Kg OS SID was administered until the normalization of serum proteins electrophoresis, which occurred seven months later. A deltamethrin-impregnated collar (Scalibor Intervet Italia/MSD Animal Health S.r.l., Italy) was recommended and it was almost constantly used by the dog.

Periodical follow-up were performed during the following months, twice a month for the first 3 months, once a month for the next 3 months, every three months in the next year, and, then, twice a year. No microfilariae were detected in peripheral wet blood films at any time.

During the follow-up, clinical and laboratory findings remained stable except for relapses of clinical leishmaniasis, treated with allopurinol alone or combined with antimoniate. During the latter relapse, the dog showed a sudden bilateral keratoconjuncivitis sicca, irreversible in the left eye (Schirmer tear test I: 0 mm/min; reference range: 15-22 mm/min) (Figure 1). Besides this, the dog has been in good health (9-9.5 Kg, BCS = 7-7.5/9) for more than three years.

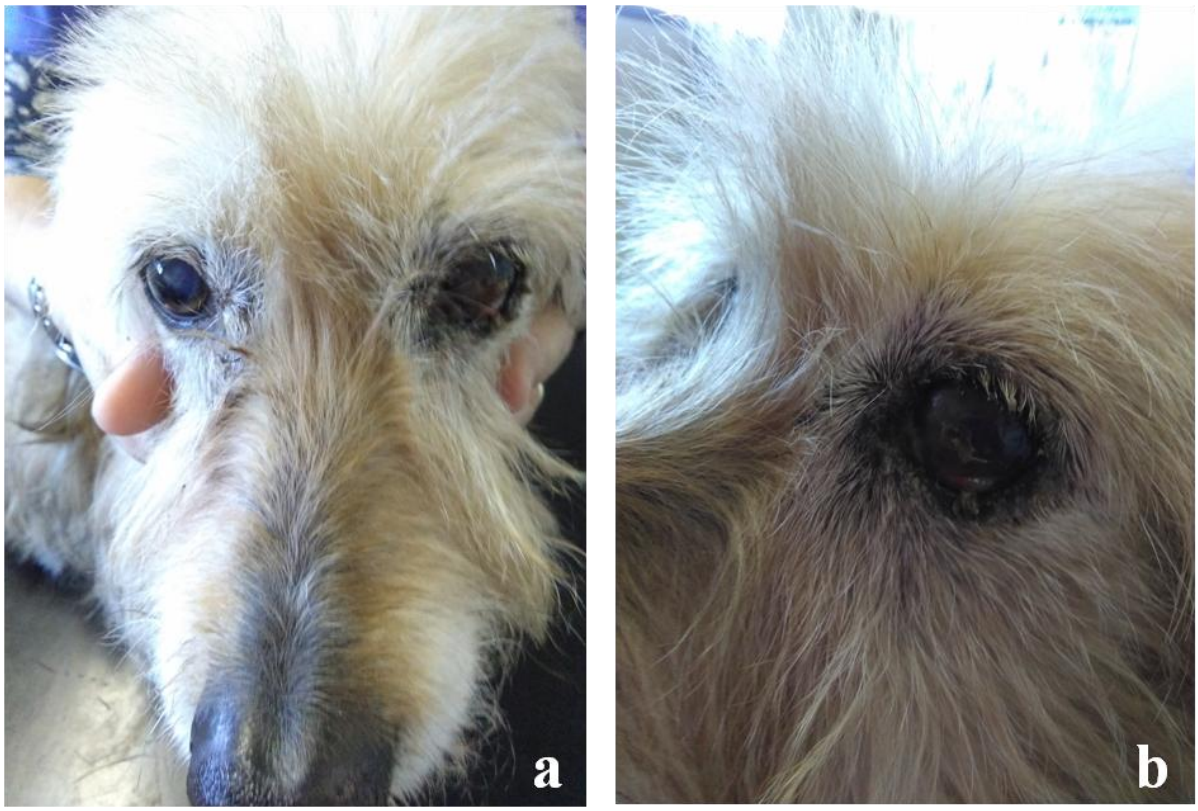


Figure 1. Bilateral keratoconjuncivitis sicca (**a**); in **b** a detail of the left eye

3.1.2 Discussion

The dog described in this report was diagnosed with aplastic pancytopenia on the basis of severe anaemia, leucopoenia and thrombocytopenia in peripheral blood, and of a bone marrow hypocellularity with >75% of the hematopoietic space replaced by fat, according to Brazzel and Weiss (2006).

Hypoplastic or hypocellular myelodysplastic syndrome (HMDS) was considered within the differential diagnosis, but it was considered unlikely because dysplastic changes were not observed in any of the cell lines, macrocytosis (a common finding in humans diagnosed with HMDS) was not observed, and blast cells were absent. Differential diagnosis between aplastic anemia and HMDS is still challenging – even in humans, where more advanced diagnostic techniques are available –, especially when dysplastic cells are difficult to detect due to marked hypocellularity of specimens and karyotipic abnormalities are not found. Immunohistochemistry and flow-cytometry has been reported to help in distinguishing between the two disorders (Serio et al., 2014; Young et al., 2006).

Serologic and molecular assays were positive for several infectious vector-borne agents, almost one of which (*Ehrlichia canis*), known to be responsible of aplastic anemia.

The pathogenic consequence of vector-borne co-infections for dogs is not well known and scarcely investigated (Baneth, 2014). The best known is the co-infection of *B. canis* with *E. canis* that is considered to determine a more severe anaemia in affected dogs (Zandvliet et al., 2004).The co-infection with multiple tick-borne pathogens can be related either to bites of several ticks carrying simultaneously different microorganisms or to the well-known possibility that a single tick species is a vector for more than one pathogen (Baneth, 2014; Kordick et al., 1999).

Ehrlichia canis, transmitted by *R. sanguineus*, is the primary cause of canine monocytic ehrlichiosis (CME). It is able to induce bone marrow aplasia, pancytopenia and high mortality in the chronic phase of the disease (Mylonakis et al., 2004). The conditions that may trigger

myelosuppression in CME have to be clearly understood, but it seems to be that breed-associated defects in cell-mediated immunity, strain variation of the pathogen, the nature of the host immune response, co-infection with other vector-borne pathogens or the development of myelofibrosis can be involved in the pathogenesis (Mylonakis et al., 2004). Recently, it has been suggested that in the myelosuppressive phase of the disease, myelofibrosis does not play a significant role unlike iron deficiency occurs, which may also exacerbate the anaemia (Mylonakis et al., 2004). A plasma cell hyperplasia is frequently recorded in the bone marrow of dogs with chronic CME but not in the acute phase. Presumably, these plasma cells represent an intramedullary immunological response secondary to the chronic infection (Mylonakis et al., 2004).

Anaplasma phagocytophilum, transmitted by *Ixodes ricinus*, is the agent of granulocytic anaplasmosis, a zoonotic disease. In the present report, *A. phagocytophilum* DNA was not detected and because of the serological cross-reaction of this species with *A. platys* and *E. canis* (Pennisi et al., 2012; Boozer and McIntire, 2003), the positive serologic test results to this organism may be considered accidental, even in view of its low prevalence in Sicily (Pennisi et al 2012; de la Fuente et al., 2006). Moreover, although *A. phagocytophilum* is known to cause transient peripheral pancytopenia, it has not been documented to cause aplastic pancytopenia in dogs, unlike in humans in which its hematopoietic marrow localization has been related to cytokines release, such as tumour necrosis factor (TNF)-alpha and interleukine-1, able to modify the bone marrow microhabitat and to determine apoptosis and impaired maturation of stem cells (Raza, 2000).

The role of *Babesia canis*, transmitted by *R. sanguineus*, remains uncertain, because it is generally responsible for haemolytic anaemia, but immunologic destruction can also contribute in the pathogenesis of the anaemia (Taboada and Merchant, 1991).

The possible influence of the rickettsiae of the spotted fever group (*Rickettsia conorii*, transmitted by *R. sanguineus*, cross-reacts with all those in the group) and of

Acanthocheilonema (*Dipetalonema*) *reconditum*, a filarial parasite of subcutaneous tissues and muscle fasciae, transmitted by fleas and lice, on the development of aplastic anaemia is still no clear. In humans, it has been documented a possible co-evolution of aplastic anaemia triggered by chronic filarial infestation by *Wuchereria bancrofti* (Hemachandran et al., 2003; Srinivas, 2009). In the case described in this study, a really high number of circulating microfilariea have been found for several weeks. This could have represented one of the component at the basis of the development of aplastic anaemia in this dog or could have been only coincidental. Therefore, further studies has to be carried out in order to evaluate the real influence of filarial infection in the pathogenesis of the disease.

Leishmania infantum, the protozoan agent of zoonotic visceral leishmaniasis, is transmitted by phlebotomus sand flies and potentially by ticks (Baneth, 2014). It is able to induce haematological abnormalities, sometimes with involvement of bone marrow that can be even heavily parasitized in dogs developing disease. Thrombocytopenia and non-regenerative anaemia are frequent clinicopathological signs. In bone marrow of leishmaniotic dogs megakaryocytic and erythroid dysplasia, and erythrophagocytosis have been detected, probably related to an increased number of marrow macrophages producing high levels of TNF-alpha and interferon (INF)-gamma (Manna et al., 2006). Moreover, a combined pathological effect of *E. canis* and *L. infantum* have already been described in previous studies (Mekuzas et al., 2009). Anyway, beyond the direct action of the individual pathogens, the multiple infections may have resulted in a serious and debilitating condition, that could have contribute in the bone marrow suppression.

In the present case, it is impossible to rule out other known causes of myelosuppression, such as drugs, toxins, or exposure to radiation (Brazzel and Weiss, 2006), because the patient was a stray dog with an unknown history. A probably pregnancy or late abortion may have contributed to the severity of the initial haematological pattern. After two blood transfusions and an ineffective stimulation with anabolic steroids, the treatment with erythroid and

granulocytic lines stimulation factors (erythropoietin and filgrastim, respectively) was proposed to the owner that refused because too expensive over a poor prognosis.

Considering the possible immune-mediated nature of bone marrow hypoplasia, we opted for immunosuppressive treatment with prednisolone. Considered the slight haematological improvement, it was decided to add cyclosporine, a T-cell selective immunosuppressant drug without mielosuppressant effect. Cyclosporine selective effect is due to a direct inhibition on the expression of nuclear regulatory proteins, resulting in reduced T-cell proliferation and activation (Archer et al., 2011; Young et al., 2006).

The autoimmune pathogenesis of aplastic anaemia is multifactorial and still not completely understood. Several laboratory and clinical studies have provided evidence that it seem likely to be related to a deregulated interaction between immune system and marrow hematopoietic stem cells, resulting in ineffective haematopoiesis due to immune-mediated apoptosis of normal hematopoietic progenitors. The damage of these cells is mediated by increased numbers of cytotoxic T cells (CTL), expressing Th1 cytokines, especially CD8 cells releasing IFN-γ (Dolberg and Levy, 2014; Young et al., 2006). Immunosuppressive therapy using cyclosporine is actually a common treatment strategy for humans with idiopathic aplastic pancytopenia, able to improve bone marrow activity and solve pancytopenia (Dolberg and Levy, 2014; Young et al., 2006). The efficacy of cyclosporine for human aplastic pancytopenia has been mainly related to the presence of activated CD8-positive cytotoxic T cells (Rosenfeld et al., 1995).

In the present report, cyclosporine induced a significant increase in CBC just after a few days. The improvement was more rapid in the red cell line (with a fast increase in reticulocytes value) than in the other cell lines. The precise mechanism of action could not be clarified because markers of CD4 and CD8 lymphocyte activation were not evaluated in this patient.

The clinical and haematological decline observed after the initial stabilization was likely to ascribe to a worsening of leishmaniasis due to the immunosuppressive therapy. Treatment

with metronidazole, a drug with anti-leishmanial activity (Noli and Auxilia, 2005), was not enough to control the disease. A good response, instead, was observed after the treatment with antimonials, originally not administered because they could increase the risk of cyclosporine nephrotoxicity (Dolberg and Levy, 2014). Despite this risk, no signs of toxicity were observed during and after the treatment period. Allopurinol was given only after cyclosporine was stopped because it might modify its plasma levels (Gorrie et al., 1994).

Finally, the reduction until the disappearance of peripheral blood microfilariae occurred during cyclosporine treatment, whereas the previous treatment with selamectin gave no positive results. This finding might be coincidental, although in humans and mice infected with lymphatic filariasis it has been hypothesized that CD4+ T cells indirectly trigger their own apoptosis by secreting significant quantities of IFN-γ, resulting in the induction of high levels of nitric oxide, and the subsequent elimination of effectors T cells. Lymphocyte apoptosis could contribute to the parasite persistence associated with the microfilaraemic state (Jenson et al., 2002). Because immunosuppressive effect of cyclosporine on T-cell results in significant decreases in IFN-γ expression (Archer et al., 2011), the reduction of microfilaraemia could be related to the treatment. Besides its immunosuppressive properties, cyclosporine possesses an anti-parasite activity against many protozoa and helminths. Although, it is not completely known how the drug exerts these anti-parasite effects, many filarial parasites possess a cyclosporine-sensitive cyclophilins, a family of enzymes which have been shown to act as catalysts of protein folding and also to bind cyclosporine (Ma et al., 1996).

In the present report, the good responsiveness to cyclosporine treatment confirms the underlying immune pathophysiology of canine aplastic pancytopoenia, similarly to what observed in humans (Young et al., 2006). Although the disease has a guarded prognosis, the dog in this report underwent a complete haematological recovery without ever relapsing. In conclusion, cyclosporine should be considered as a immune suppressive treatments in dogs

with bone marrow disorders associated with CVBDs, together with concomitant treatment of the underlying infectious agent.

3.2 Outbreaks of Canine Adenovirus (CAV-1) infection in four shelter dogs in Sicily

Canine adenoviruses, CAV-1 and CAV-2, are members of the genus *Mastadenovirus*, family Adenoviridae, and are closely related antigenically and genetically (75% identity at the nucleotide level) (Davison et al., 2003). However, despite their genetic and antigenic similarities, they show different haemoagglutination pattern and cell tropisms. CAV-1 replicates in the vascular endothelium, and hepatic and renal cells, whereas CAV-2 in the respiratory tract and, less commonly, in the intestinal epithelia (Decaro et al., 2008). Canine adenoviruses have been known as pathogens of the dogs since several decades. CAV-1 infection was first described in 1930 (Green et al., 1930), when it caused a epizootic encephalitis of foxes, and was definitively isolated in 1940 (Decaro et al., 2008). CAV-2, instead, was first isolated in 1961 from dogs with laryngotracheitis (Swango et al., 1969). Infections by CAV have been detected in several mammalian species, such as dogs (Muller et al., 2010), red foxes (Walker et al., 2016; Thompson et al., 2010), wolves (Stephenson et al., 1982) and coyotes (Gese et al., 2004), whereas CAV antibodies have been detected also in other carnivores and marine mammalians, like black bears, fishers, polar bears, wolves, walruses (Philippa et al., 2004), Stella sea lions (Burek et al., 2005) and Eurasian river otter (Park et al., 2007).

Canine Infectious Hepatitis (ICH) is a systemic disease caused by CAV-1. The virus replicates in the vascular endothelial cells and hepatocytes causing acute necro-haemorrhagic hepatitis, that is more severe in younger animals. The transmission of the virus occurs by the oro-nasal way for direct contact or, indirectly, after exposition to infected saliva, urine, faeces or respiratory secretions. Asymptomatic dogs can spread the virus in the environment and convalescent dogs can eliminate the virus though nasal excretions and the urine for up to 9

months after the recovery of clinical signs. The incubation period is of 4 to 6 days after indirect and 6 to 9 days after direct contact. During the viremic phase, that lasts 4 to 8 days, the virus reaches the target organs (lungs, spleen, liver and kidneys). Initially, organ damages are caused by the cytotoxic activity of the virus; however, after approximately one week, the immune system reacts by producing antibodies that cause a worsening of tissue damages due to the deposition of immune complexes.

Canine Adenoviruses can survive up to several months in the environment at temperature between 4° and 20° C and pH between 6.5 and 8. On the contrary, they are quickly inactivated at temperature higher than 60° C and after the exposition to UV rays. CAV-1 is resistant to freezing and to organic lipophilic detergents, whereas it is sensible to 1-3% sodium hypochlorite (bleach).

The disease usually occurs in young, not vaccinated, animal, but also older dogs can be affected. The hyperacute form of ICH determines sudden death within a few hours after the onset of clinical signs. The acute form is characterized by biphasic fever (> 40° C), with a first peak that last 1 or 2 days which may be followed by a second peak in the most severely affected dogs. Other clinical signs are weakness, sensorium depression, anorexia, tachycardia, tachypnoea, icterus and gastrointestinal signs, such as vomit and diarrhoea. Abdominal pain and distension are the results of the accumulation of haemorrhagic fluid in the intestinal bowls and of hepatomegaly. Less common clinical signs reported in dogs with ICH are haemorrhagic diathesis, haemorrhage of mucous membranes and of the skin, epistaxis, neurological signs (hypersalivation, ataxia and seizures) that are caused by vascular damage of the Central Nervous System (CNS) and respiratory distress, caused by laryngitis, tracheitis and, eventually, aspiration pneumonia. The corneal opacity (the so-called “blue eye”) and interstitial nephritis usually occur in animals that manage to overcome the acute phase of the disease, generally 1 to 3 weeks after the remission of the other clinical signs, and they are both caused by the deposition of immune complexes. Ocular lesions are caused by a

hypersensitivity phenomenon type III with the deposition of immune complexes and chemotaxis of inflammatory cells in the anterior chamber of the eye (Carmichael, 1964). The following damage to the corneal endothelium determines the development of corneal oedema and uveitis, with blepharospasm, photophobia and serous ocular discharge.

Haematological and haematochemical common findings in dogs with ICH are leucopoenia, with reduction of both lymphocytes and neuthophils, and increase of liver enzymes (aspartate aminotransferase, AST, alanine aminotransferase, ALT, and alkaline phosphatase, ALP). Coagulation disorders, such as thrombocytopenia, prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT), and increased levels of fibrin degradation products (FDPs), like the D-dimers, may occur secondary to Disseminated Intravascular Coagulation (DIC) (Wigton et al., 1976). On urine analysis, severe proteinuria can be found, due to immunomediated glomerulonephritis.

Diagnosis of ICH can be suspected based on history, clinical signs and, eventually, post-mortem and histopathologic findings. However, virus isolation on Madin Darby canine kidney (MDCK) cells, and polymerase chain reaction (PCR) on ocular swabs, urine faeces and post-mortem samples, are essential to achieve a definitive diagnosis. Immunofluorescence (IF) can be used to detect viral antigens but, as well as virus isolation, does not allow to distinguish between CAV-1 and CAV-2. Serology has a lower diagnostic value because also vaccinated dogs would result to be positive.

Treatment of ICH is symptomatic and supportive, in order to correct dehydration, DIC and to prevent secondary bacterial infections.

Prevention of adenoviruses infection in dogs, has been widely realized through vaccination with CAV-2 attenuated vaccines, that ensure cross-protection also for CAV-1. The protection seems to last for three years after the second administration (Gore et al., 2005). Several attempts have been made in order to formulate a vaccine containing CAV-1. However, the

inactivated forms came out to need repeated injections (Miller et al., 1980), whereas the modified-live ones were associated with interstitial neprhitis and corneal opacity.

Systematic vaccination of dogs has considerably reduced circulation of CAVs in canine populations, although severe outbreaks can be still observed in countries in which CAV vaccines are not used routinely or as a consequence of uncontrolled importation of dogs from endemic areas. ICH infection has been described in Switzerland (Müller et al., 2010), Canada (Wong et al., 2012), Alaska (Caudell et al., 2005), in Portugal (Duarte et al., 2014) and in Italy (Decaro et al., 2007). A few reports of co-infection of CAV-1 together with canine distemper virus (CDV), in Japan (Kobayashi et al., 1993), CDV, canine parvovirus (CPV) infection, canine infectious tracheobronchitis and toxoplasmosis, in Brazil (Headley et al., 2015), and with coronavirus (CCV), in Italy (Pratelli et al., 2001), have been described. ICH infection has rarely been described in adult vaccinated animals. However, in the north of Italy 7.8% of asymptomatic and vaccinated dogs tested for CAV-1, came out to be positive at PCR (Balboni et al., 2014). Traditionally, CAV-1 infections in vaccinated dogs have been related to inappropriate vaccination or to interference with maternal antibodies. However, two recent studies have described ICH caused by possible new variants of adenovirus (Balboni et al., 2017; Wong et al., 2017), which could explain the onset of the disease even in vaccinated dogs due to a vaccine failure or to a greater virulence of the virus. These reports prove that, despite the widespread vaccination has dramatically reduced the incidence of CAV-1 infection in dogs, the disease has not been completely eradicated and should always be considered in the differential diagnosis when consistent clinical signs occur, even in adult vaccinated dogs.

The aim of this report was to give a contribution to the limited literature available on CAV-1 infection in dogs, by describing two outbreaks of Infectious Canine Hepatitis (ICH), alone or together with canine parvovirus, in four puppies from two different areas of Sicily (Italy).

3.2.1 Cases presentation

This report describes two different outbreaks of Canine Adenovirus (CAV-1) infection in two different areas of Sicily (Italy).

The first outbreak involved three mix-breed puppies from the same litter (cases 1, 2 and 3), housed in the municipal kennel of Palermo (Sicily, Italy). The puppies were one male and two females of 2 months old (body weight: mean 4.43±0.5 standard deviation, SD), that had been lived in the kennel since they were10 days old. They had been fed with artificial milk for 10 days and, then, they were weaned. When the outbreak occurred, the puppies were still not vaccinated and the immunological status of the mother was unknown. The first puppy was found death in the kennel, without any previous clear clinical sign. After two weeks, the other two puppies showed a slight serous conjunctivitis and diarrhoea, that were treated symptomatically with fluid therapy and antibiotic, with almost complete remission of clinical signs. However, after other two weeks, more severe gastrointestinal signs (vomit and diarrhoea) re-occurred. Moreover, one of the two puppies, showed severe neurological signs (seizures) and died within a few hours. The day after, also the last puppy showed similar a clinical condition, with fever (40.3 °C), lethargy, pale mucous membrane (CRT > 2 seconds), severe vomit and diarrhoea, and seizures. Penicillin G benzathine-diidro streptomycin (Rubrocillina Vet®, Intervet Productions Srl, Aprilia, LT, Italy) 5 mg/kg intramuscularly (IM), two boluses of Ringer Lactate Solution (Ringer Lattato S.A.L.F.,S.A.L.F. S.p.A. Laboratorio Farmacologico, Cenate Sotto, BG, Italy) of 20 mL/kg each, intravenously (IV), and vitamin B complex (Stimulfos®, Teknofarma SpA, Torino, TO, Italy) 2 mL/10kg of body weight, IV, were immediately administered, but the puppy died after 2 hours. Based on history and clinical signs, the most likely differential diagnosis were canine parvovirus (CPV), canine coronavirus (CCV), canine distemper (CDV) and CAV-1. To achieve a definitive diagnosis, the puppy was sent to the Istituto Zooprofilattico Sperimentale (IZS) of

the Sicily “A Mirri”, for necroscopic and virologic examinations, that were performed within two hours after death.

The necroscopic examination showed mild serous abdominal effusion, enlarged and congested liver with several red to grey spots on the hepatic parenchyma (Figure 1), and congestive-haemorrhagic lesions on the peritoneum, spleen, kidneys, intestine, lungs and pericardium.

Figure 1. Hepatomegaly with congested lived, icterus and several red spots on the surface of the hepatic parenchyma

During the necroscopy, samples from heart, spleen, lungs, kidneys, brain, liver and intestinal bowls, were collected for virological examinations. Moreover, a sample of faeces was collected directly from the intestinal lumen for coprological and virological examinations through Enzyme Linked Immunosorbent Assay (ELISA) technique, for protozoa (*Giardia* spp., coccidia), tapeworms (Dipylidium caninum, Mesocestoides spp., Taenia spp.) and nematodes, and for Rotavirus and Coronavirus, respectively.

Samples collected from the different organs were homogenized in *Minimum Essential Medium* (MEM) synthetic cell culture media (Minimum Essential Medium Eagle, Sigma-Aldrich S.r.l. Milan, Italy), and analyzed with Polymerase Chain Reaction (PCR) for canine parvovirus (CPV), canine distemper virus (CDV) and canine adenovirus (CAV-1 and CAV-2). PCR technique used for the detection of adenovirus (Hu et al., 2001), allowed to differentiate between CAV-1 and CAV-2.

For virus isolations, an aliquot of each homogenate was inoculated on cell cultures specific for each viral pathogen tested. In particular, a monolayer of *Madin-Darby Canine Kidney Cells* (MDCK, Sigma-Aldrich S.r.l. Milan, Italy) has been used for the isolation of adenovirus. Every day, the culture has been checked in order to detect any eventual cytopathic effect caused by the virus. Immunofluorescence (IF) was performed from the second culture on MDCK cells, by using a Anti-Canine Adenovirus (CAV) polyclonal antiserum conjugated to fluorescein isothiocyanate (VMRD, Veterinary Medical Research and Development, Pullman, Washington, USA). Moreover, on the same culture, PCR for adenovirus was repeated. Nucleic acids have been extracted with commercial kits for DNA (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germania) and RNA (High Pure RNA Isolation Kit, Roche, Monza, Italy). For the research of CAV, 5 µL of DNA were amplified, by using the kit GoTaq DNA Polymerase (Promega Corporation, Madison, Wisconsin, USA), in a volume of 50 µL containing 1x GoTaq Reaction Buffer (Promega Corporation, Madison, Wisconsin, USA), 0,1

µL of each primer, 0,2 µL of a solution of Deoxynucleotide Triphosphates (dNTP mix, Promega Corporation, Madison, Wisconsin, USA) and 1,25 U of GoTaq DNA Polymerase.

Virological examinations (ELISA) performed on faeces gave negative results for Rotavirus and Coronavirus, whereas coprological exams were positive for *Toxocara canis.*

Virological tests on the specific organs gave negative results for CPV, CDV and CCV. The PCR for adenovirus detection, gave negative results for CAV-2 and positive results for CAV-1 in all the organs analyzed (heart, spleen, lungs, kidneys, brain, liver and intestine) (Figure 2). Moreover, all the organs were positive for CAV-1 after virus isolation on MDCK, with a cytopathic effect produced by the virus already in the second culture (Figure 3 and 4). The virus isolated from the cellular culture, was identified through IF and PCR.

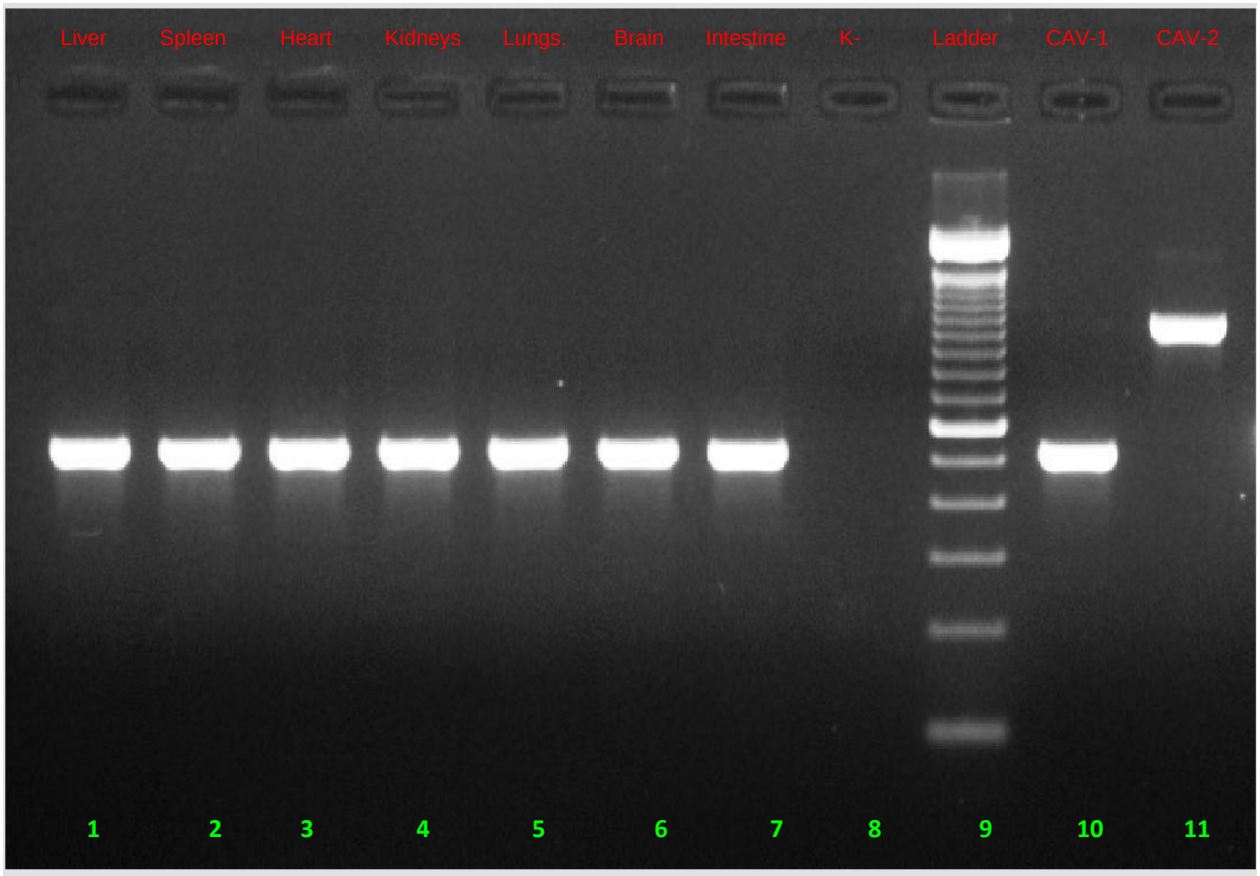


Figure 2. PCR for the detection of CAV-1 and CAV-2 on different organs. The first 7 stripes represent each specific organ (liver, spleen, heart, kidneys, lungs, brain and intestine), strip number 8 is the negative control, the 9th is the scale in bmap, and the 10th and 11th are the positive controls for CAV-1 and CAV-2, respectively

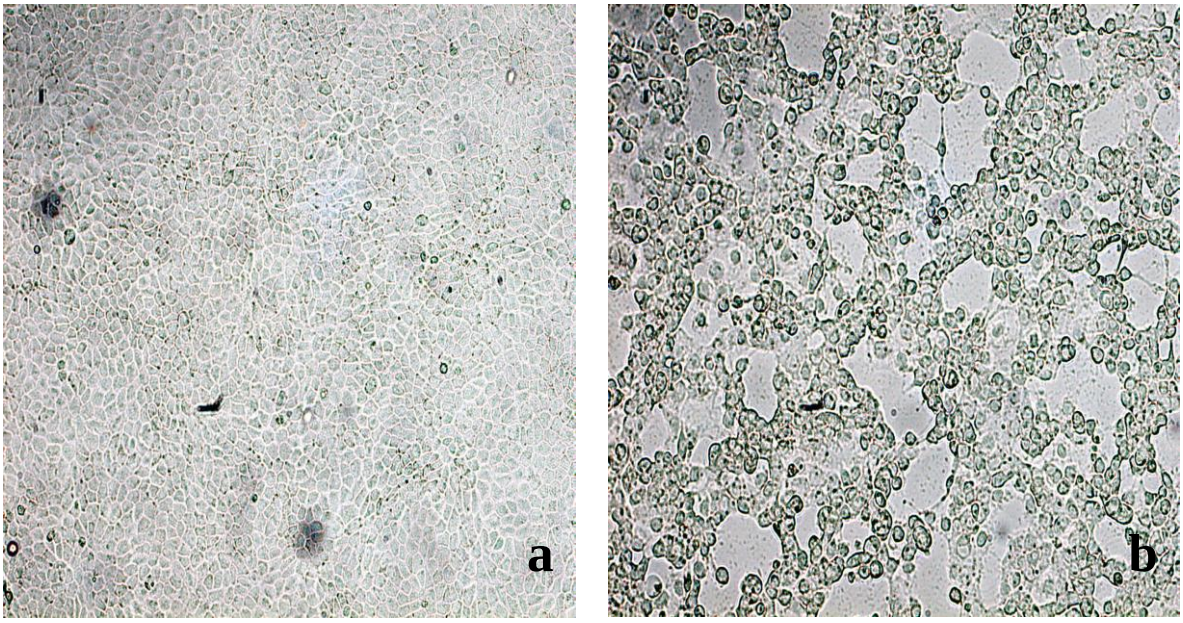


Figure 3. Monolayer of *Madin-Darby Canine Kidney Cells* (MDCK), before (**a**) and after the cytopathic effect of CAV-1 (**b**) (100x)

The second outbreak involved a female mix-breed puppy of 4 months old and10 kg of body weight (case 4), housed in a dog shelter in Messina (Sicily, Italy). The dog had been admitted to the shelter 5 days before and previous history was unknown.

The dog was referred to the Veterinary Teaching Hospital of the University of Messina because of anorexia, lethargy, vomit (1 to 3 times per day) and diarrhoea since 2 days. On general physical examination, the dog showed lethargy, pale and sticky mucous membranes with CRT of 3 seconds, tachycardia (160 beats/minute) and weak pulse, tachypnoea (35 breaths/minute), rectal temperature of 39.3 °C, a dehydration of 7%. and weight loss (Body Condition Score, BCS, 3/9). Traces of bloody and smelly faeces were found on the thermometer after measurement of rectal temperature. On chest auscultation, no abnormalities in lungs and heart sounds were found. On abdominal palpation, abdomen was slightly painful, intestinal bowls were mildly full of fluid and gas, and intestinal borborygmi were increased. Immediately after abdominal palpation, vomit and diarrhoea, with bloody and smelly faeces, occurred (Fecal Score, FS, 5; normal between 2-3). Because of lethargy, tachycardia and weak pulse, the dog was considered to be in shock. A venous catheter was immediately placed on a cephalic vein and a bolus of 20 mL/kg of Ringer Lactate Solution was administered. In the mean time, a blood sample was collected from the jugular vein for laboratory examinations. A microhaematocrit tube was filled with a drop of whole blood to assess the microhaematocrit and total solids (TS). For haematology, 1 mL of blood was place in a tube with EDTA. Two mL were placed in a tube with a clot activator gel and were centrifuged, after 15 minutes from blood collection, at 3000 rpm for 10 minutes, in order to collect a sample of serum for haematochemical examinations. One drop of whole blood was used for the assessment of blood glucose by mean of a portable glucose meter (Glucocard, Menarini, Firenze, Italy). An ELISA snap test for Parvovirus (Snap Parvo Test, IDEXX, Westbrook, Maine, USA), was performed on a rectal swab. A sample of serum and faeces were sent to the IZS of Sicily “A. Mirri”, for CPV detection.

On admission (Day 1), TS (5.1 g/dL; laboratory range: 6.5-7.5 g/dL) and the microhaematocrit (27%) were decreased. On haematology, slight microcitic hypochromic anaemia (Red Blood Cells, RBC: 5.3 x 10^6/µL, laboratory range: 5.65-8.87 x 10^6/µL; Packed Cell Volume, PCV: 29.6%, laboratory range: 37.3-61.7%; Haemoglobin, Hgb: 10.5 g/dL, laboratory range: 13.1-20.5 g/dL; Mean Corpuscolar Volume, MCV: 55.5 fL, laboratory range: 61.1-73.5 fL; Mean Corpuscolar Haemoglobin, MCH: 19.7 pg, laboratory range: 21.2-25.9 pg), severe thrombocytopenia (Platelets, PLT: 18 x 10^3/µL, laboratory range: 148-464 x 10^3/µL). Thrombocytopenia was confirmed by platelet estimation on a blood smear stained with May Grünwald-Giemsa, that showed also the presence of schistocytes. Kidney and liver values on haematochemical examination were within the laboratory ranges. The ELISA Snap Test for CPV was positive. Based on clinical findings and on the positive ELISA Snap Test for CPV, a diagnosis of canine parvovirus infection was made.

After the resolution of the shock, maintenance and correction of dehydration fluid therapy was started at 5 mL/kg/hour for the first 24 hours. Antibiotic therapy with amoxicillin and clavulanic acid (Synulox®, Pfizer Italia Srl Div. Vet., Rome, Italy) 12.5 mg/kg subcutaneously (SC) once per day (SID) and metronidazol (Metronidazolo S.A.L.F, S.A.L.F. S.p.A. Laboratorio Farmacologico, Corate Sotto, BG, Italy) 10 mg/kg two times per day (BID) intravenously (IV) in 20 minutes, were started to prevent secondary bacterial infection and the translocation of intestinal bacteria. Ranitidine (Zantadine®, Ceva, Agrate Brianza, MB, Italy) 1 mg/kg SC BIB and metoclopramide (Vomend®, Eurovet Animal Health BV, Bladel, The Netherlands) 0.2 mg/kg IV BID were started to reduce gastric acidity and nausea, respectively, and enteric nutrition with gastrointestinal soft food was provided. As soon as dehydration was corrected, fluid therapy was switched to maintenance plus the correction of ongoing losses.

General clinical condition of the dog improved during the first 3 days of treatment, with normalization of rectal temperature (38-38.7 °C). The vomit persisted, with 1 to 3 episodes

per day. Since the 4th day, rectal temperature increased again to 39.9 °C, despite the antibiotic treatment. Moreover, the haematological profile repeated the same day, showed a worsening of the anaemia (RBC: 5.12 x 10^6/µL; PCV: 27%), of the thrombocytopenia (PLT: 15 x 10^3/µL) and the onset of leucopoenia (White Blood Cells, WBC: 5 x10^3/µL; laboratory range: 5,05-16,76 x 10^3/µL).

The dog remained almost stable since the 7th day of treatment, when a sudden and severe worsening if clinical conditions occurred, with severe depression of the sensorium, anorexia, vomit, diarrhoea, distal oedema of the limbs and haemorrhagic diathesis with mucosal petechiae. Moreover, compared to admission, the dog showed a significant reduction of body weight (BW, 8.5 kg; BCS 3/9), despite the constant enteral nutrition and fluid therapy. Haematological profile repeated on day 7 showed a further worsening of the anaemia (RBC 4.83 x 10^6/µL; PCV: 25.7%), leucopoenia with lymphopoenia (lymphocytes: 0.95 x 10^3/µL; laboratory range: 1.05-15.10 x 10^3/µL), eosinopoenia (eosinophils: 0.02 x 10^3/µL; laboratory range: 0.06-1.23 x 10^3/µL), monocytosis (monocytes: 1.14 x 10^3/µL; laboratory range: 0.16-1.12 x 10^3/µL) and bands. Reticulocytes were low (2.9 x 10^3/µL; laboratory range: 10-110 x 10^3/µL), so the anaemia was considered to be not regenerative.

The same day, the dog had several episodes of vomit with dark blood, melena with watery faces and showed dark urine and worsening of the distal oedema. On physical examination the dog was severely depressed, mucous membranes were icteric (CRT of 3 seconds), the pulse was weak with a frequency of 110 bpm, and thachypnoea (respiratory rate: 35 breaths/minute) was found. A sample of blood was collected from the lateral saphenous vein, because of the thrombocytopenia and the suspect of clotting problems, and was divided into one tube with the clot activator gel and in one tube with sodium citrate for the evaluation of clotting times. A prolonged bleeding was observed after blood collection. Haematochemical profile showed a severe increase in liver enzymes (ALT: 810 U/L; laboratory range: 8-75 U/L; AlkP: 337 U/L; laboratory range: 46-337 U/L; γ Glutamil Transferase, γGT: 4U/L; laboratory range: 0-2

U/L: total bilirubin: 1,2 mg/dL; laboratory range: 0-0,8 mg/dL), hypoproteinemia (total protein, TP: 4.4 g/dL; laboratory range: 4.8-7.2 g/dL), hypoalbuminemia (albumin 1.7 g/dL; laboratory range: 2.1-3.6 g/dL), low phosphate (P: 3.7 mg/dL; laboratory range: 5.1-10.4 mg/dL). Total calcium was also low (6.8 mg/dL; laboratory range: 7.8-12.6 mg/dL), probably due to the low albumin. Clotting times were prolonged (Prothrombine Time, PT: 19 seconds; laboratory range 1-17 sec; Activated Partial Thromboplastin Time, APTT: 173 sec; laboratory range: 72-102 sec), showing a clotting deficit. Based on clinical and laboratory findings, a Disseminated Intravascular Coagulation (DIC) was strongly suspected. A Tick Born Disease (TBD) or a dicumarinic poisoning could not be completely ruled out even if, especially the second, was considered unlikely based on anamnesis. Considering the liver failure, a concomitant adenovirus infection was added to the differential diagnosis list. Since the dog had severe vomit, doxycycline could not be administered, and it was decided to administer imidocarb diproprionate (Carbesia®, Intervet Italia Srl, Milano, MI, Italy) 4 mg/Kg IM once, for the suspect of TBD. Phytomenadione (Konakion®, Roche SpA, Segrate, MI, Italy) 5 mg/Kg IV BID was started because of the possible dicumarinic poisoning and for the severe liver disease. For the latter, also Acetylcysteine (Fluimucil®, Zambon, Bresso, Italy), 70 mg/Kg/die IV, and glutathione (TAD 600®, BIOMEDICA FOSCAMA IND.C.F. Spa, Rome, Italy) 15 mg/kg IV SID were started. Stanozolol (Stargate®, Acme Srl, Cavriago, RE, Italy) 25 mg IM once, was administered in order to try to stimulate to bone narrow. Although a blood and/or a plasma transfusion would have been necessary to treat the severe anaemia and the low total proteins, and clotting disorders, respectively, blood products were not available at that moment. For this reason, it was decided to administer 20 mL/kg of albumin 5% (Octalbin® 25%, diluted with 1:4 ratio in NaCl 0.9%, under sterile conditions) at the rate of 5 mL/kg/h and 5 mL/kg of hydroxyl ethyl starch (Voluven®, Fresenius Kabi Italia S.r.l., Isola della Scala, VR, Italy) at the rate of 2 mL/kg/h, for the oncotic properties.

The following day (day 9) clinical condition improved with normalization of rectal temperature (38.5 °C) and no episodes of vomit and diarrhoea. On the contrary, the haematological profile showed a further worsening of the anaemia (RBC: 2.81 x 10^6/µL; PCV: 14.3%; Hgb: 5.9 g/dL; reticulocytes: 0.6 x 10^3/µL), lower neutrophils and eosinophils values (neutrophils: 2.63 x 10^3/µL; eosinophils: 0.01 x 10^3/µL), increased monocitosis (monocytes: 1.80 x 10^3/µL), while lymphocytes were within the laboratory range (1.56 x 10^3/µL). Platelets were lower than the lower value detected by the machine.

On the 10^{th} day, mucous membranes were still icteric with some petechiae. Serum levels of total protein and albumin were still low (4.3 and 1.5 g/dL, respectively) despite the administration of albumin. On the same day, a bag of fresh frozen plasma DEA 1.1 negative became available and was administered. The rate of administration was gradually increased until the total dose of 20 mL/kg was administered in 6 hours.

Eighteen hours after the end of plasma transfusion (Day 12), clinical conditions and laboratory findings of the dog were considerably improved. The pulse was strong, 104 bpm, RR was 20/minute, CRT was 2 seconds and TS were 6 mg/dL, while the microhaematocrit was 12.5%. The worsening of the haematological profile was probably due to a dilution phenomenon. However, reticulocytes and platelets were increased compared to previous days (5.5 and 2 x 10^3/µL, respectively).

During the following days of hospitalization, general clinical conditions of the dog improved; rectal temperature and CRT came back to physiological values, faeces and urine became of normal colour and consistence, and the dog started to eat on her own.

After 2 weeks of hospitalization (Day 14) a little corneal opacity localized in the inferior medial area of the left cornea was found that, within the following morning, generalised to the whole eye (Figure 4). On ophthalmologic examination, it was found a diffuse corneal oedema and no corneal ulcerations on fluorescein test, and a topical treatment with tobramycin/dexamethasone drops (Tobradex®, Alcon Italia S.p.A., Milan, Italy) three times

per day (TID) was started. On the same day, blood and urine samples and rectal and conjunctival swabs were sent to the IZS of Sicily "A. Mirri", for adenovirus detection.

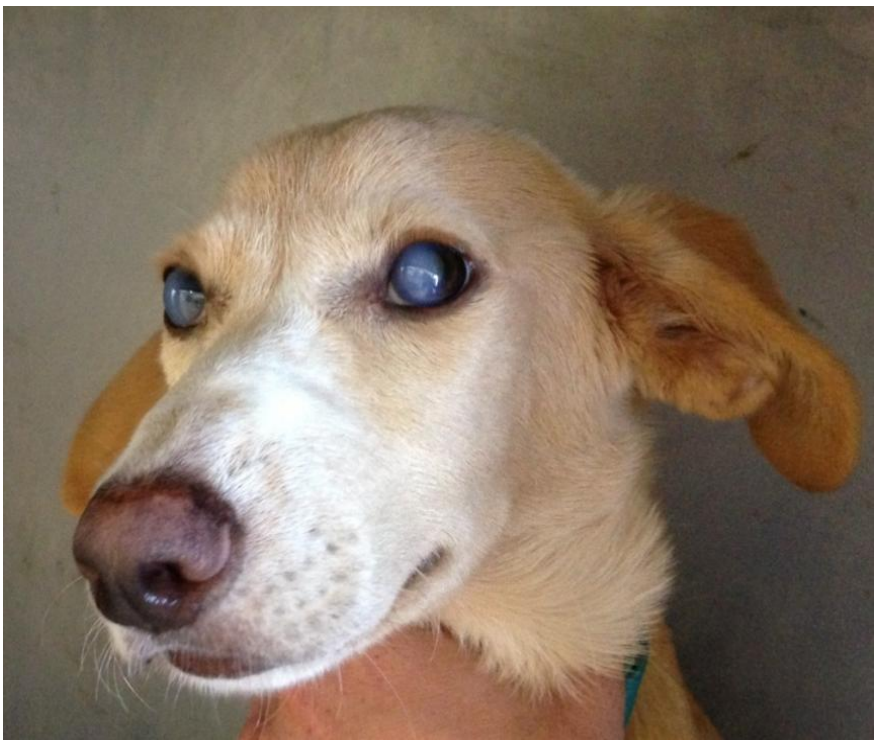


Figure 4. Bilateral and diffuse severe corneal oedema ("so-called "*blue eye*") in the fourth puppy (case 4) described in this report

On day 16, the clinical condition of the dog further improved, no other episodes of vomit had occurred within the previous days and faeces consistence gradually came back to normal. Haematological profile also improved (RBC: 3.45 x 10^6/µL; PCV: 20.7%; Hgb: 7.2 g/dL; MCV: 60 fL; reticulocytes: 176 x 10^3/µL; PLT: 110 x 10^3/µL; WBC: 5.35 x 10^3/µL; neutrophils 2.79 x 10^3/µL). However, ocular signs did not improve. For this reason, indomethacin drops (Indom®, Alfa Intes, Casoria, NA, Italy) and sodium chloride 5% in gel

(Edenorm®, Sooft Italia S.p.A., Montegiorgio, FM, Italy) four times per day, were added to ocular treatment, and the topic antibiotic was switched to fluocinolone acetonide/neomycin sulphate (Iridex®, Ceva Vetem S.p.A., Agrate Brianza, MB, Italy), four times per day.

The dog was discharged the same day with the prescription to continue ocular and hepatic treatment (Epatopasta Plus®, DNR Srl, Palazzo Pignano, CR, Italy) for other 10 and 20 days, respectively.

On the follow-up after the end of the prescribed ocular treatment, the dog was clinically stable and ocular lesions had considerably improved. Only a little area of corneal opacity was left in the inferior medial part of the right cornea. Haematological and haematochemical profiles, showed a further improvement of all blood cells (RBC: 3,88 x 10^6/µL; PCV: 22,1%; Hgb: 8,1 x g/dL; MCV: 57 fL; reticulocytes: 222,3 x 10^3/µL; PLT: 385 10^3/µL; WBC: 6,05 x 10^3/µL; neutrofili: 3,06 x 10^3/µL) and of liver function (BUN: 10 mg/dL, laboratory range: 7-29 mg/dL; creatinine: 0.7 mg/dL, laboratory range: 0.3-1.2 mg/dL; ALT: 272 U/L; AST: 77 U/L, laboratory range: 0-50 U/L; ALP: 551 U/L).

Virological test came out to be positive for CPV (on the first serum sample collected on admission) and for CAV-1, confirming the diagnosis of ICH. All the other viruses tested were negative.

Haematological and haematochemical profiles of the fourth puppy, during all the hospitalization period, are summarized in Table 1.

Table 1. Haematological and haematochemical profiles of the fourth puppy (case 4), during the hospitalization period and on follow-up

Parameter (unit)	Laboratory range	Day of hospitalization							
		1°	*4°*	*7°*	*9°*	*10°*	*12°*	*16°*	*Follow-up*
TS (g/dL)	6,5-7,5	5.1	/	/	/	/	6.0	7.6	/
RBC (x 10^6/µL)	5,65-8,87	5.3	5.12	4.83	2.81	/	2.05	3.45	3,88
PCV (%)	37,3-61,7	29.6	27	25.7	14.3	/	10.8	20.7	22,1
Hgb (g/dL)	13,1-20,5	10.5	10.4	10.1	5.9	/	4.2	7.2	8,1
MCV (fL)	61,1-73,5	55.5	52	/	/	/	52.7	60	57
MCH (pg)	21,2-25,9	19.7	18.5	/	/	/	/	/	/
Reticulocytes (x 10^3/µL)	10-110	/	15	2.9	0.6	/	5.5	176	222,3
WBC (x 10^3/µL)	5,05-16,76	6.76	5.00	5.13	6.00	/	4.04	5.35	6,05
Neutrophils (x 10^3/µL)	2,95-11,64	/	/	3.02	2.63	/	1.72	2.79	3,06
Linfocytes (x 10^3/µL)	1,05-5,10	/	/	0.95	1.56	/	/	/	/
Eosinophils (x 10^3/µL)	0,06-1,23	/	/	0.02	0.01	/	/	/	/
Monocytes (x 10^3/µL)	0,16-1,12	/	/	1.14	1.80	/	/	/	/
PLT (x 10^3/µL)	148-464	18.0	15.0	14.7	0	/	2	110	385
ALT (U/L)	8-75	/	/	810	/	/	/	/	272
AST (U/L)	0-50 U/L	/	/	292	/	/	/	/	77
AlkP (U/L)	46-337	/	/	337	/	/	/	/	551
γGT (U/L)	0-2	/	/	4	/	/	/	/	/
Total bilirubin (mg/dL)	0-0,8	/	/	1,2	/	/	/	/	/
TP (g/dL)	4,8-7,2	/	/	4,4	/	4,3	/	/	/
Albumin (g/dL)	2,1-3,6	/	/	1,7	/	1,5	/	/	/
Phosphate (mg/dL)	5,1-10,4	/	/	3,7	/	/	/	/	/
Total Ca^{2+} (mg/dL)	7,8-12,6	/	/	6,8	/	/	/	/	/
PT (sec)	11-17	/	/	19	/	/	/	/	/
aPTT (sec)	72-102	/	/	173	/	/	/	/	/

3.2.2 Discussion

Nowadays, infections caused by adenoviruses are considered to be very rare. Systematic vaccination of owned dogs has gradually reduced the incidence of CAV infections, since it has been considered to be almost eradicated. However, some reports exist in the veterinary literature describing sporadic outbreaks of the infection in different areas of the world (Duarte et al., 2014; Headly et al., 2013; Wong et al., 2012; Caudell et al., 2005). Historically, outbreaks of CAV infection have been associated with stray dogs or puppy imported from eastern countries, due to too early vaccination, when the title of Maternal Derived Antibodies (MDA) is still too high.

Wild animals could also be responsible of the circulation of the virus in the environment. Especially CAV infections in red foxes and wolves have been frequently described (Walker et al., 2016; Thompson et al., 2010) all around the world. However, only one report in the north of Italy described the presence of CAV-1 and CAV-2 in wild red foxes (Balboni et al., 2013).

The south of Italy is well-known to have still the problem of several free ranging stray dogs, which act like a reservoir of several infectious diseases (Otranto et al., 2017). Decaro et al. (2007) have described four outbreaks of CAV-1 infection in Apulia and Basilicata regions (south of Italy). Three outbreaks were observed in shelter dogs, while the fourth was observed in two pure-breed dogs imported a few days before from Hungary. Some years before, another report described a co-infection of CAV-1 and canine calicivirus (CCV) in puppies housed in a shelter in Apulia region (Pratelli et al., 2001). In both studies, infected animals showed one or more clinical signs compatible with ICH (fever, lethargy, anorexia, vomit, diarrhoea, respiratory distress, mucopurulent conjunctivitis and neurological signs) and all the sample collected underwent virus isolation and molecular examinations for CAV and other common enteric pathogens of the dogs. CAV-1 was detected as the sole cause of clinical signs in only one outbreak, whereas it was associated with CDV, CCV and CPV in the others.

In the north of Italy, one study has reported that among 51 asymptomatic dogs tested with PCR for adenoviruses, 7.8% and 58.8% were positive for CAV-1 and CAV-2, respectively, even in adult and vaccinated dogs (Balboni et al., 2014). The findings of this study seem to suggest that vaccination protects from the development of the disease, but could not protect from infection and shedding of CAV-1 and CAV-2. In fact, from sequencing of the isolated CAVs, it was noticed that the circulating strain is genetically stable and similar between dogs and wild animals, so reducing the possibility of the existence of a new strain resistant to vaccination (Balboni et al., 2014).

The present report describe four cases of adenovirus (CAV-1) infection alone (cases 1, 2 and 3) or together with CPV infection (case 4) in two different areas of Sicily (South Italy). No previous reports have been found in the veterinary literature on the outbreak of ICH in Sicily. As already described in previous studies, also the clinical cases described in this report were young, not vaccinated puppies that showed hyperacute to acute onset of gastrointestinal signs in all the four puppies, while neurological and ocular signs occurred only in one puppy each. ICH led to death within a few hours from the onset of clinical signs the three puppies housed in the shelter. On the contrary, the fourth puppy, which was hospitalized in a Intensive Care Unit, managed to recover from the disease after a long period of hospitalization, despite a co-infection with CPV. The positive outcome of the fourth puppy was probably due to the intensive monitoring and treatment received, compared to the other three that were only roughly treated. However, also the older age of the survivor could have been linked to a more complete maturation of the immune system, so determining a greater ability to “fight” against the pathogens.

Disseminated Intravascular Coagulation has been reported as a possible and severe complication in dogs affected by several pathological conditions, such as CPV and CAV infections (Müller et al., 2010). Also in the cases described in this report, the DIC could have occurred, determining the death of the first three puppies (cases 1, 2 and 3). Moreover, the last

puppy showed clinical (haemorrhagic diathesis and mucosal petechiae) and laboratory abnormalities (schistocytes, severe thrombocytopenia and prolonged clotting times) compatible with DIC. The constant consumption of clotting factors and platelets, and the contact between red blood cells and fibrin, that occur during the pathogenesis of DIC, may cause haemorrhages and morphological alteration of the red blood cells, so determining the formation of schistocytes. Unfortunately, it was not possible to determine the fibrin degradation products (FDPs) and the D-dimers, and to perform a thrombelastography (TEG), that would have confirm the diagnosis of DIC.

Considering that initially blood products were not available, human albumin was administered in order to correct the severe hypoproteinemia and hypoalbuminemia, with only slight improvements. On the contrary, the following administration of fresh frozen plasma, allowed to achieve an almost complete normalization of total protein, albumin and clotting time.

The onset of ocular lesions and corneal opacity has been described in the 66.7% of survivors (Decaro et al., 2007). As already described in previous studies, no ocular lesions occurred in the puppies that die in the acute phase, and the classic form of “blue eye”, due to the corneal oedema, appeared only during the recovery phase of the disease for the deposition of immune complexes (Scatozza, 2006). The prompt beginning of ocular therapy have probably led to the almost complete resolution of ocular lesions, despite the severe initial alterations. Because of the severe leucopoenia, it was decided to start only a topic treatment with corticosteroid to reduce immune complexes deposition, instead of a systemic one. Moreover, the use of the osmotic gel and indomethacin, that has shown to have good anti inflammatory activity even in immune mediated flogosis, both in the eyes and in other organs (Sagawa et al., 1996), has probably determined the resolution of corneal oedema.

In this report, virus isolation on MDCK cells provided a cytopathic effect in all the sample tested (100% sensitivity). However, this technique is not specific for CAV and PCR is always

required for a definitive diagnosis and in order to differentiate between CAV-1 and CAV-2 infections.

This report would be a contribution to the small literature on CAV-1 infection in dogs. Together with previous ones (Pratelli et al., 2001; Decaro et al., 2007; Balboni et al., 2014), this report demonstrates that CAV-1 is rare but still circulating in the dog population, despite constant vaccination, and can be responsible of severe and often fatal clinical conditions especially in animal shelters and kennels. In fact, in these situations, the close contact within the animals can make easier the diffusion of the disease. Although, the mortality rate of ICH is usually low (10 to 30% of infected animals), co-infections with other viral or bacterial pathogens have been described to make the evolution and the prognosis of the disease worse. However, this report shows that even single infection with CAV-1 can be a very life-threatening condition, especially in young dogs that live in overcrowded shelters, if a prompt intensive treatment is not started.

For these reasons, CAV-1 infection should always be included in the differential diagnosis for every conditions characterized by gastrointestinal, neurologic and ocular signs. Moreover, even if ocular signs ("blue eyes") are pretty indicative of ICH, it has to be considered that they occurred only during the recovery phase of the disease. Therefore, virological examinations for CAV should be performed every time the disease is suspected, in order to achieve a definitive diagnosis and start an early treatment.

In conclusion, despite the vaccination with CAV-2 is still the most effective way to control the diffusion of the disease (AAHA, Canine Vaccine Guidelines of the American Animal Hospital Association), an early diagnosis and an intensive treatment can increase the possibility of a positive outcome in sick dogs.

V. PAIN EVALUATION AND ANALGESIA

4.1 Attitudes, knowledge and opinions of Italian veterinary practitioners towards pain and analgesia in dogs and cats: regional North-South scenarios

The word “pain” is derived from the Latin *peone* that means *penalty* and from the Greek *poine* which refers to *punishment*. Defining the painful experience has been a longstanding, difficult and important issue. Aristotle first described pain as originating from specific types of stimulation, including heat, cold, toxins, and crush, which leads to acute awareness of the need to escape. More recently, increasing scientific evidences have shown that animals can experience or feel pain as humans do. Moreover, animals have been always used in research to understand the patho-physiological processes of pain sensation in humans and to test drugs intended for the use in human pain therapy. For this reason, it has to be assumed that, if a disease or surgical procedure causes pain in human, at least the same degree of pain has to be felt by animals. Several scientific studies have demonstrated that the components of the nociceptive system are basically the same between human and mammalian animals. Moreover, no differences in pain perception have been found in animals of different ages, even in new-born animals.

Traditionally, it has been believed that some pain persisting into the postoperative period may be helpful to encourage immobility and, in turn, healing and recovery. Similarly, acute pain occurring at the area of injury in the trauma patient can serve to help protecting the body part or system, minimizing further injury. However, it is now well-known that pain causes several changes of the physiological status of the patient, causing negative metabolic and endocrine

effects and decreasing the time and the possibility of healing. Most important, pain leads to patient suffering. According to Maslow's hierarchy of needs model, proposed by Abraham Maslow in 1934, most of human (and perhaps animal) behaviour is attributed to a perpetual drive to meet specific needs. Maslow considered not all needs to be of equal importance and proposed that humans will not exhibit behaviours designed to meet higher order needs (e.g., personal achievement) unless basic needs (e.g., food, water, shelter) are first adequately met. Maslow arranged the needs that may drive human behaviour into a pyramid with the most important or essential needs (physiological needs) at the bottom. Some researchers have expanded Maslow's hierarchy into sub-hierarchies. Physiological needs are not all given equal priority, and pain is on the second stair of the pyramid, after the need of air (Figure 1) (Mellema and McIntyre, 2014).

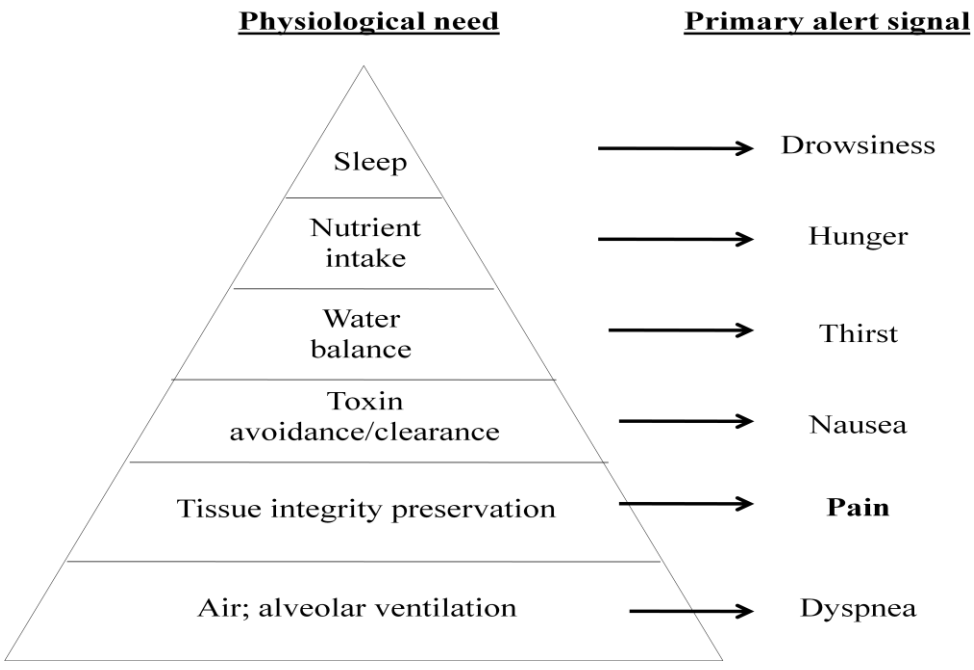


Figure 1. Sub-hierarchy of physiological needs according to Maslow's hierarchy of needs and the primary alert signals related to each need

By accepting that animals feel pain, we can move on to the more important issues of defining, assessing, and learning how to prevent and alleviate their pain.

The International Association for the Study of Pain (IASP) has proposed a definition of pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. In addition, the IASP stated that “the inability to communicate in no way negates the possibility that an individual is experiencing pain and is in need of appropriate pain relieving treatment” (International Association for the Study of Pain task force on taxonomy, 1994). More recently, the AVMA’s position stated that “animal pain and suffering are clinically important conditions that adversely affect an animal’s quality of life” (AVMA adopts position regarding animal pain, 2001). In other words, pain is not only the nociception itself but it is especially the conscious experience of nociception, which is only partly determined by the stimulus-induced activation of afferent neural pathways. All these definitions express the concept that pain is a conceptual sense, always subjective and unpleasant, and that it is classified as an emotional experience. By definition, pain is a subjective event and cannot truly be measured in an accurate fashion by an outside observer. Pain experience, even after the same painful stimulation, is never felt with the same intensity by two different individuals. Moreover, pain cannot be measured exactly and it has to be accepted as the patient says it is. The perception of pain and response to a noxious stimulus are determined not only by the degree of injury but also by the individual’s unique experience. The reason why different people may report different degrees of pain after the same painful stimuli could be also due to a genetic basis, related to a different morphology, number and distribution of the opioid receptors (La Forge et al., 2000). Pain assessment becomes inherently more difficult in nonverbal humans and in veterinary patients due to the obvious limitations in verbal communication, with attempts to anthropomorphize the animal’s behaviour, potentially increasing the degree of error in our assessment.

The veterinary practitioner has a moral and ethical obligation to alleviate animal pain and protect them from suffering. Although there is a constantly increase in knowledge and emphasis on pain management as the awareness of the complexity of pain pathways and the potential for long-term detrimental effects in veterinary patients have increased, pain evaluation and therapy are still, most of the time, lacking in veterinary practice.

The causes of this limited attitude against pain have been recognized to be ideological, technical and related to a poor ability in recognizing pain and lack of knowledge on pharmacokinetics, pharmacodynamics and side effects of the most common used analgesics (Hugonnard et al., 2004). Since a few years ago, pain has been thought to be a protective mechanism to ensure immobility after a surgical procedure, so to prevent complications related to an excessive movement of the patient. Moreover, some people think that animals do not feel pain as human do or, at least, they tend to underestimate pain management in their patients. However, these issues are gradually been overcoming because of ethical considerations and of the increased awareness of owners and veterinarians about this topic. Several studies have been already carried out in different countries on veterinarians attitude towards pain assessment and therapy.

The first investigation on the administration of analgesics in companion animals was carried out in the USA on dogs (243) and cats (15) undergoing different types of surgeries (limb amputations, limb-sparing bone cancer resection, thoracotomy, cervical vertebra instability repair and humeral fracture repair) (Hansed and Hardie, 1993). In this study, 40% of dogs (96 out of 243) and only 1 cat was treated with analgesics in the post-operative period. Dogs hospitalized in the Intensive Care Unit (ICU) were more likely to receive pain medications, which were prescribed mainly by interns and residents. Vocalization was the clinical sign most used to interpret pain. Another survey carried out in Canada in 1994 (Dohoo and Dohoo, 1996a) showed that the 49.5% of the veterinary practitioners who answered the questionnaire were analgesic users (veterinarians reported to give analgesics to 50% or more of dogs or cats

after abdominal surgery) and butorphanol was the most commonly used opioid in both dogs and cats. Moreover, post-operative pain in cats undergoing orthopaedic surgery was found to be underestimated compared to dogs (80% and 70% of dogs and cats, respectively treated with analgesics) (Dohoo and Dohoo, 1996a). Factors that were identified as distinguishing analgesic users from nonusers were pain perception score, concern regarding the use of potent opioid agonist, gender, age, year of graduation, practice size and presence of animal health technologists (Dohoo and Dohoo, 1996b). The same survey, repeated in 2001, showed a remarkable increase in analgesic use, with 62% of veterinarians giving analgesic peri-operatively (Hewson et al., 2006a). This findings showed that the attitude of veterinary practitioners in Canada improved, probably due to a combination of greater awareness of animal pain and more knowledge on analgesics properties and side effects. However, even after 7 years, 12% of veterinarians assessed not to use any analgesic and butorphanol was again the most used analgesic in Canada. In 1996, a survey regarding attitude to postoperative analgesia in dogs has been carried out in the United Kingdom (UK) (Capner et al., 1999). The results obtained from this survey showed that most of the veterinarians thought that the surgical procedures considered in the questionnaire were potentially painful and only 6% claimed that surgery was not usually sufficiently painful to warrant analgesic therapy (Capner et al., 1999). However, despite this, about 30% of the questioned veterinarians considered that pain could guarantee animal immobility after surgery so reducing possible complications. Unlike the others study already mentioned, in the British survey the most commonly used opioid was the buprenorphine (Capner et al., 1999). A survey on attitudes of veterinarians towards pain assessment and analgesia has been carried out in France (Hugonnard et al., 2004). A questionnaire was designed and sent to all veterinarians involved in small animal practice of the Rhône-Alpes region. The questionnaire was filled in by 189 veterinary practitioners, mainly young (aged between 23 and 35 years) (62%) and female (53.8%), but the level of concern about pain issued was not influenced by age and gender. Other surveys

have been carried out in Finland (Raekallio at al., 2003), Brazil (Lorena et al., 2014) and Switzerland (Perret-Gentili et al., 2014). Like the above mentioned studies, the results of these studies showed that pain scores assigned to cats were lower than to dogs and differences in the frequency of pain relief between different clinical and surgical conditions were shown. In the Swiss study (Perret-Gentili et al., 2014), 88% of the participants answered favourably to pain medication and the 64.1% stated that the main reason for the administration of analgesics was the relief of a painful sensation. Also in this study, butorphanol and buprenorphine were more used compared to pure agonist opioid. In the Brazilian survey, instead, morphine was the most used opioid, together with tramadol and NSAIDs (Lorena et al., 2014).

To summarize, in almost all the above mentioned studies women and younger veterinary practitioners assigned higher pain scores to different clinical and surgical conditions and were more likely to use analgesics. Demographic data collected in the mentioned studies are reported in Table 1. Moreover, cats were less likely to receive pain relief compared to dogs and the assessment of pain was made most of the time through clinical and behavioural evaluations, whereas pain scales were almost never used. Butorphanol was one of the mainly used analgesic both in older and recent studies. However, considering that butorphanol is known to be only mildly analgesic and that its duration of activity in dogs and cats is only 2 hours (Camargo et al., 2011), even analgesic users may have not provided adequate post-operative analgesia (Hewson et al., 2006a; Hewson et al., 2006b). Moreover, when the correlation between the presence of veterinary technicians and use of analgesic was investigated, veterinarians were more likely to perform a better pain relief when at least a nurse was present in the veterinary practice (Hewson et al., 2006). In two surveys carried out on veterinary technicians attitude on pain assessment and management performed in Canada (Dohoo and Dohoo, 1998) and in the UK (Coleman and Slingsby, 2007), pain scores given by veterinary nurses were higher for all procedures than those of veterinary surgeons in previous studies (Capner et al., 1999; Dohoo and Dohoo, 1996a). Moreover, if the English study of

veterinary surgeons was made by other researchers and several years older, the Canadian ones were made by the same authors and were also closer in time. Veterinary nurses were also found to be more aware of the importance of pain scales for pain assessment compared to veterinarians. These findings underlie the importance of nurses in veterinary practices and, especially, the need to have an operator specialized in pain evaluation and pain therapy. Just to mention, other surveys in veterinary medicine have been carried out in the UK to assess the attitude of veterinary practitioner on chronic pain in dogs (Bell et al., 2014) and of owners and veterinarians on the use of NSAIDs in dogs with osteoarthritis (Belshaw et al., 2016).

In Italy pain management has been underestimated for several years both in human and in veterinary medicine. In human medicine, for example, a big survey carried out in 128 Italian ICU (Bertolini et al., 2002) has shown that, most of the time, pain was not adequately treated even in severely ill postoperative patients. The data on 918 patients collected in different ICU showed that 36.3% of them did not receive any analgesic treatment during the first 48 postoperative hours. Moreover, among the patients who received analgesics, 42% had only a single bolus per day. The main reasons given by the physicians for each administration on opioid were mechanical ventilation and anxiety, while only in 54.5% of the times pain was the reason. The probability of receiving pain relief was even lower in coma patients. This finding underlie the difficulty of assessing pain in non verbal patients, that is probably the main limitation in animals. A survey carried out among the Neonatal Intensive Care Units (NICUs) in Italy (Lago et al., 2005) showed a percentage of administration of analgesics varying from 1% for injections to 67% per chest tube insertion. The results of this survey demonstrated an inadequate management of pain in NICUs in Italy, even if the percentage of analgesics users was higher than in the study on adult ICU patients (Bertolini et al., 2002) This could be due to the fact that the study on neonatal pain was carried out a few years later and that the topic of pain in infants and children is source of a greater empathy. The reasons mentioned as limitations for the use of analgesia were risk/benefit ratio, poor confidence on analgesics

properties, lack of training in pain control and difficulty with assessing pain without clear verbal communication.

In veterinary medicine, a survey has been recently done on veterinarians attitudes and knowledge on abdominal visceral pain in dogs in Italy (Catanzaro et al., 2016). The results of the questionnaire showed that main reasons for abdominal pain among Italian veterinarians were diseases of the gastrointestinal tract (35.2%), pancreas (15.4%) and reproductive system (11.1%), while only 8.7% indicated the peritoneum as a source of pain. Seventy four percent of veterinarians did not use any pain score system and 16% only sometimes, even if 73% stated to be often able to diagnose abdominal pain. Clinical signs commonly used to assess abdominal pain were hunched back, reluctance to move, praying position, anorexia and dysorexia, while behavioural changes were cited only a few times. Respondents were mainly young (57% under 40 years old) and female (66%). However, unlike other surveys, female and male scores assigned for different pathological conditions were not statistically different, with the exception of metritis and pyometra that received higher scores from female veterinarians. The most commonly used analgesics were opioids (40%) and among them, even in this case, the most cited was the butorphanol. Tramadol was also frequently used (20%) and this could be due to the lack of a specific prescription for opioid and of a regulatory control.

Table 1. Demographic information of the respondents of surveys on pain attitude in different countries. Number of respondents (N), gender (female [F] and male [M]), percentage of veterinarians under 40 years old and of veterinarians graduated within 10 years before each study was carried out

	Country	N	Gender		Young veterinarians (under 40)	Graduation since less than 10 years
			F	M		
Dohoo and Dohoo, 1994	Canada	275	48%	52%	59%	48%
Hewson et al., 2006	Canada	326	39%	61%	55%	43%
Capner et al., 1999	UK	958	/	66%	/	/
Hugonnard et al., 2004	France	189	30.1	69.9%	30.5% (under 35 y)	/
Raekallio et al., 2003	Finland	434	66%	34%	/	41.2%
Lorena et al., 2014	Brazil	1298	58%	42%	/	67%
Perret-Gentili et al., 2014	Switzerland	258	48.4%	48.8%	/	/
Catanzaro et al., 2016	Italy	527	66%	/	57%	51%

4.1.1 Aim of the study

The aim of this study was to evaluate the attitudes and knowledge on pain assessment and analgesia in veterinary practitioners from the South compared to the North of Italy. Considering that the previous study from Catanzaro et al. (2016) gave an overall view of pain therapy in Italy, the aim of this study was to assess if any regional variation exists and if analgesia is an adequately considered topic in the South of Italy.

4.1.2 Materials and method

A questionnaire to assess veterinarians attitude and knowledge regarding pain management and analgesia was developed. A preliminary revisions of the questionnaire was carried out by a pilot sample of 5 selected veterinary practitioners in order to assess if the meaning of the questions was clear and the same for all the responders. All the veterinarians involved in small animal practice and students of the fourth and fifth years of Veterinary Medicine courses in Italy were eligible for the survey. After being revised, the final version of the questionnaire was built using an online format (www.survio.com). The relative web link, together with a cover letter containing the information about the researcher behind the study, the aim of the survey, the time needed to fill it in and the assurance that the responder would have remained anonymous, was sent to veterinary associations of the different Italian provinces. Every association was asked to readdress the files to the veterinary practitioners of their professional registers. The study started in January 2015 with the preparation of the questionnaire and the following correction and revision by the selected testers. Questionnaires were sent out in October 2016 and data collection was closed in August 2017.

The questionnaire formulation was based on previously published survey on pain attitude in small animals (Catanzaro et al., 2015; Perret-Gentili et al., 2014; Hewson et al., 2006; Hugonnard et al., 2004) and was written in the native language of the recipients (Italian). The

questionnaire consisted of 4 sections, including 17 questions in total. At the end of the last section, an open space was left for any eventual comment or personal opinion.

This was a cross-sectional survey. The sampling carried out for this survey was a non-randomized, purposive one, because it was included in the survey only the specific population of small animal veterinary practitioners (Kelley et al., 2003).

The first Section consisted in questions regarding the demographic data of the responder. The questions were aimed at collecting information regarding the gender (female or male), age range (20 to 30, 31 to 40, 41 to 50 and 51 to 60 years old), professional level (student, PhD student, researcher, University professor, or private practitioner), main area of interest (internal medicine, surgery or reproduction), University of graduation and the city were the professional activity is carried out.

Questions regarding pain assessment were included in Section 2. The first question asked to estimate the current knowledge on evaluation and pain therapy (very low, mild, advanced and specialized). The second question was about the evaluation systems frequently used. The interviewed could answer choosing from: “none”, “clinical evaluation”, “Visual Analogue Scale (VAS)”, “Numerical Rating Scale (NRS)”, “Simple Descriptive Scale (SDS)”, “Glasgow Composite Pain Scale”, “Feline Glasgow Composite Pain Scale”, “Colorado State University (CSU) canine pain scale”, “CSU feline pain scale” and “UNESP-Botucatu”. More than one answer was possible. The third question was about the parameters most commonly used to assess pain in dogs and cats. Among the possible answers there were behavioural changes, vocalizations, decreased appetite, body tension, aggression, reaction to palpation, heart rate, respiratory rate, arterial blood pressure, decreased grooming and reaction to stimulation. The responders could give more than one answer and, for each parameter, they could choose if they use it in cats, dogs or both. In the last question of Section 2, it was asked to the interviewed to assign a verbal score (slight, mild, severe and terrible) to various pathological or surgical conditions, based on their feeling on the severity of each situation.

The conditions considered were arterial thromboembolism (ATE), bone fracture, central nervous system (CNS) diseases, cystitis, diaphragmatic hernia, gastric dilatation volvulus (GDV), hepatitis, lacerated wounds of the skin, neoplasia, ocular diseases, orthopaedic surgery, osteoarthritis, otitis, ovariohysterectomy, pancreatitis, peritonitis, pleuritis, polytrauma, pyelonephritis, spleen torsion and urethral obstruction. All the conditions were listed in alphabetical order to decrease the possible influence of the position in the list on the personal judgment. For the statistical analysis, a numerical value was attributed to each verbal score (1 for slight, 2 for mild, 3 for severe, 4 for terrible and 0 if the answer was not filled in).

The third Section was about pain therapy. The first question asked about the frequency of use of analgesics. The answer had to be chosen between “never”, “rarely (months)”, “sometimes (weeks)”, “often (days)” and “every day”. The respondents who answered “never” or “rarely (months)” had to explain the reasons of their limited use of analgesics by choosing among “difficulty in pain assessment”, “wish to limit animal movement after a surgical procedure”, “lack of knowledge on analgesic pharmacology”, “fear of side effects”, “lack of scientific data”, “belief that animals do not feel pain as humans do”, “fear of hiding the course of the disease”, “low attitude” and “cost of the analgesics”. The next two questions were about the most commonly used analgesic classes (α_2-agonist, corticosteroid, local anesthetics, NSAIDs, or opioids) and active principles (tolfenamic acid, betamethasone, butorphanol, buprenorphine, carprofen, coxibs, dexmedetomidine, dexamethasone, fentanil, ketamine, ketoprofen, medetomidine, meloxicam, methadone, methylprednisolone, morphine and tramadol), respectively. As previously, the possible answers were listed in alphabetical order and the responders could give more than one answer. For the statistical analysis, a numerical value was assigned to each specific analgesic if it was used (1) or not used (0). After these two questions, an open space was left to give the opportunity to the interviewed to justify the reasons of their choices. The last question of the section asked if the responders knew the meaning of and if they make use of the “preventive analgesia”.

The last Section was about the ways to acquire knowledge on pain management and analgesia. In particular, it was asked to the responders which were the sources of education they used (seminars/congresses, scientific articles or books). Moreover, it was asked if the responders considered their level of knowledge of analgesia satisfactory ("in no way", "not much", "enough" or "absolutely yes").

The questionnaire had to be completely fill in the first section (demographic data) and at least 80% of the questions answered to be used in the data analysis. Questionnaires filled in for less than 80% were discarded. Responders were divided in two groups, North and South, based on the province were their professional activity was carried out. Respondents of the North group were from Rome (included) to the north, while the South group was made of respondents that worked southwards to Rome (excluded).

Statistical analysis

All the data were entered in a datasheet spread (Microsoft Excel). Descriptive statistic was applied to calculate the percentage rate for each answer. When more than an answer was allowed, the overall percentage rate added to more than 100%, and the reported percentages refer to the total number of citations for each answer and not to the number of respondents. Pearson χ^2 square, Fisher's exact and McNemar chi-square tests were used in order to determine the effect of geographical area, gender and age on the degree of severity assigned to each pathological or surgical condition and on the list of analgesics used.

Statistical significance was set at $P < 0.05$. The statistical test was performed using the software STATISTICA 7 (Stat Soft Inc., USA, 2003).

4.1.3 Results

At the end of the study period, 185 questionnaires met the inclusion criteria and were collected. Among these, 90 (48.65%) were of the North group and 95 (51.35%) of the South group.

Among the respondents, 109 (58.9%) were female and 76 (41.1%) were male, 73 (39.5%) aged between 20 and 30 years, 58 (31.3%) between 31 and 40 years, while only 34 (18.4%) and 20 (10.8%) were in the 41-50 and 51-60 groups, respectively. When considering the two specific groups, in the North group 56 (62.2%) were female and 34 (37.8%) were male (Figure 2). Based on the age range, 25 (27.8%; 16 female and 9 male) were in the 20-30, 28 in the 31-40 (31.1%; 18 female and 10 male), 22 in the 41-50 (24.4%; 14 female and 8 male) and 15 (16.7%; 8 female and 7 male) in the 51-60 group, respectively. In the South group, 53 were female (56.7%) and 42 (44.2%) were male. Among them, 48 (50.5%; 28 female and 20 male) were in the 20-30 group, 30 (31.6%; 19 female and 11 male) in the 31-40, 12 (12.6%; 6 female and 6 male) in the 41-50 and 5 (5.3%; 0 female and 5 male) in the 51-60 group, respectively (Figure 3).

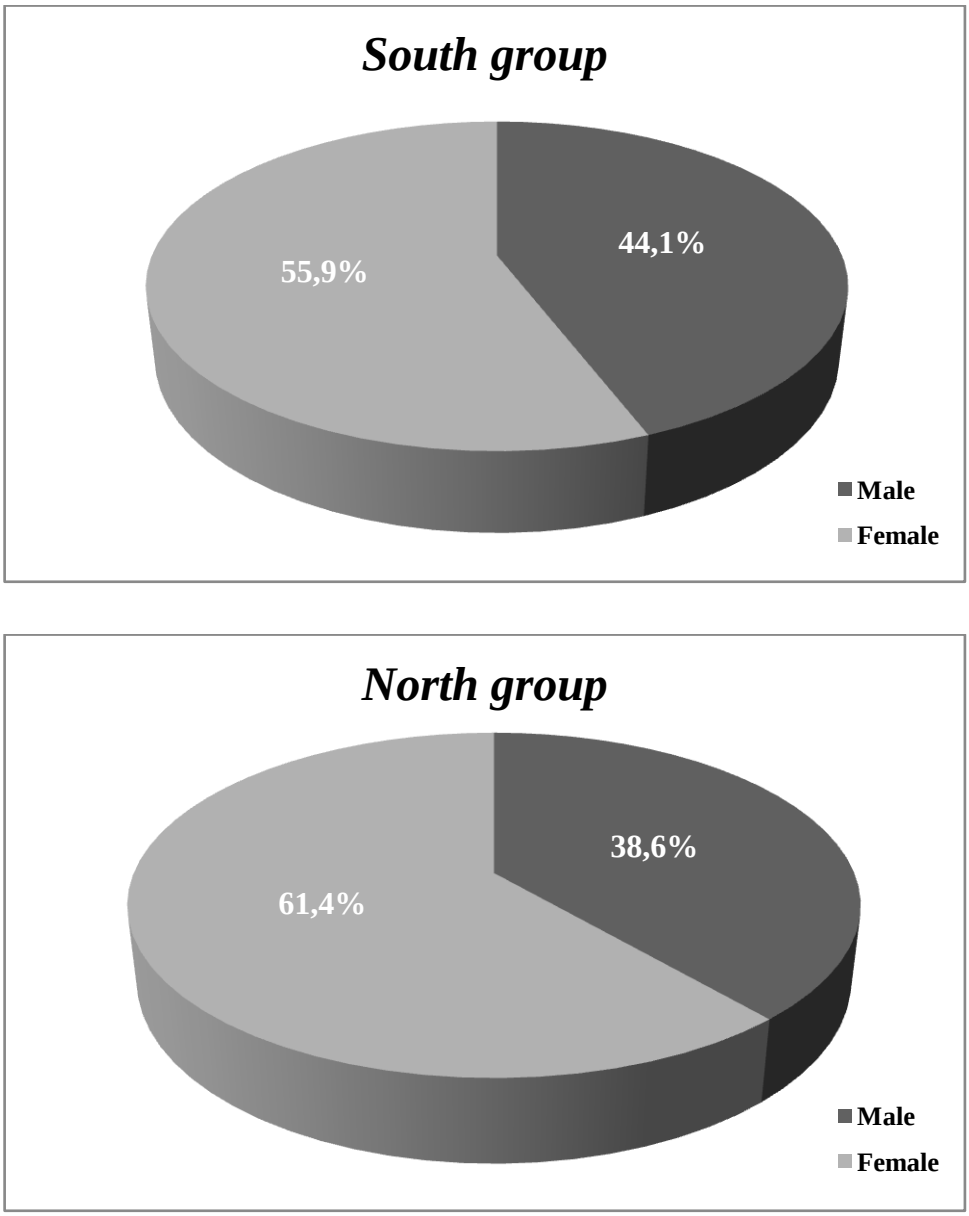


Figure 2. Percentages of female and male respondents in the South and North groups

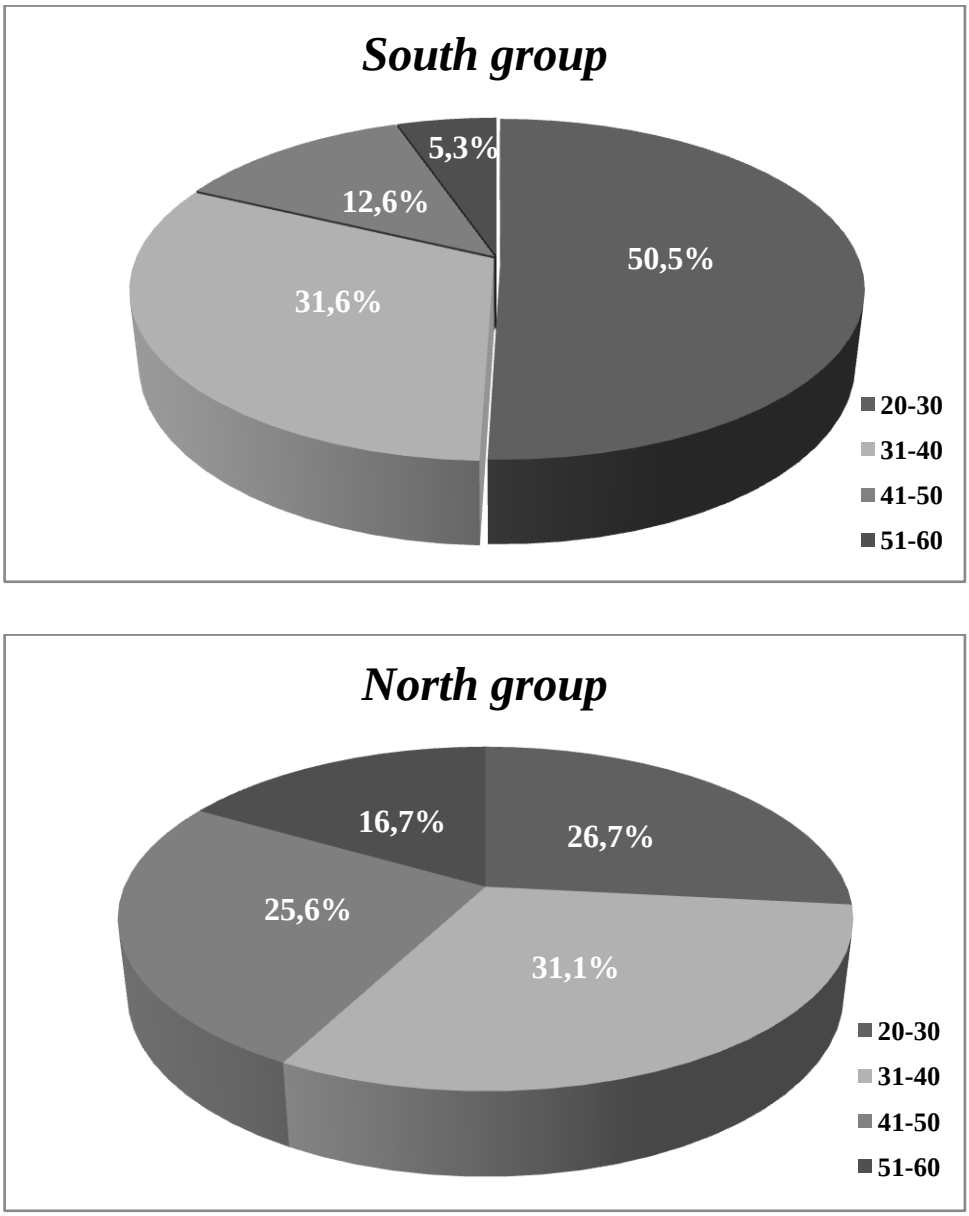


Figure 3. Age ranges (20-30, 31-40, 41-50 and 51-60 years old) of respondents in the South and North groups

The respondents were mainly private practitioners in both North and South groups (95.6% and 66%, respectively). Among the other professional levels, in the North group 2 University professors (2.2%), 1 researcher (1.1%) and 1 PhD student (1.1%) answered the questionnaire. In the South group, instead, the questionnaire was filled in by 24 students of the 4^{th} and 5^{th} year of the Veterinary Medicine course (25.5%), 7 PhD students (7.4%), and 1 researcher (1.1%). Among the North group respondents there were no students, while in the South group no University professors (Figure 4).

The main area of interest was in both groups, internal medicine (63.7% and 59.6% in the South and North groups, respectively), followed, in order, by surgery (34.1% and 36%) and reproduction (2.2% and 4.5%).

Seventy-five percent of the South group graduated at the University of Messina, 9.2% at the University of Bari, 5.3% at the Universities of Naples and Teramo, and 1.3% at the Universities of Sassari, Bologna and Perugia. In the North group, 32.2% graduated at the University of Perugia, 10% at the University of Torino, 8.9% at the Universities of Bologna and Messina and 7.8% at the University of Pisa. Other Universities of graduation of the North group respondents were Naples (5.6%), Parma and Milan (4.4%), and Bari, Padua and Camerino (2.2%).

Provinces of professional activity of both North and South groups respondents are summarized in Table 2.

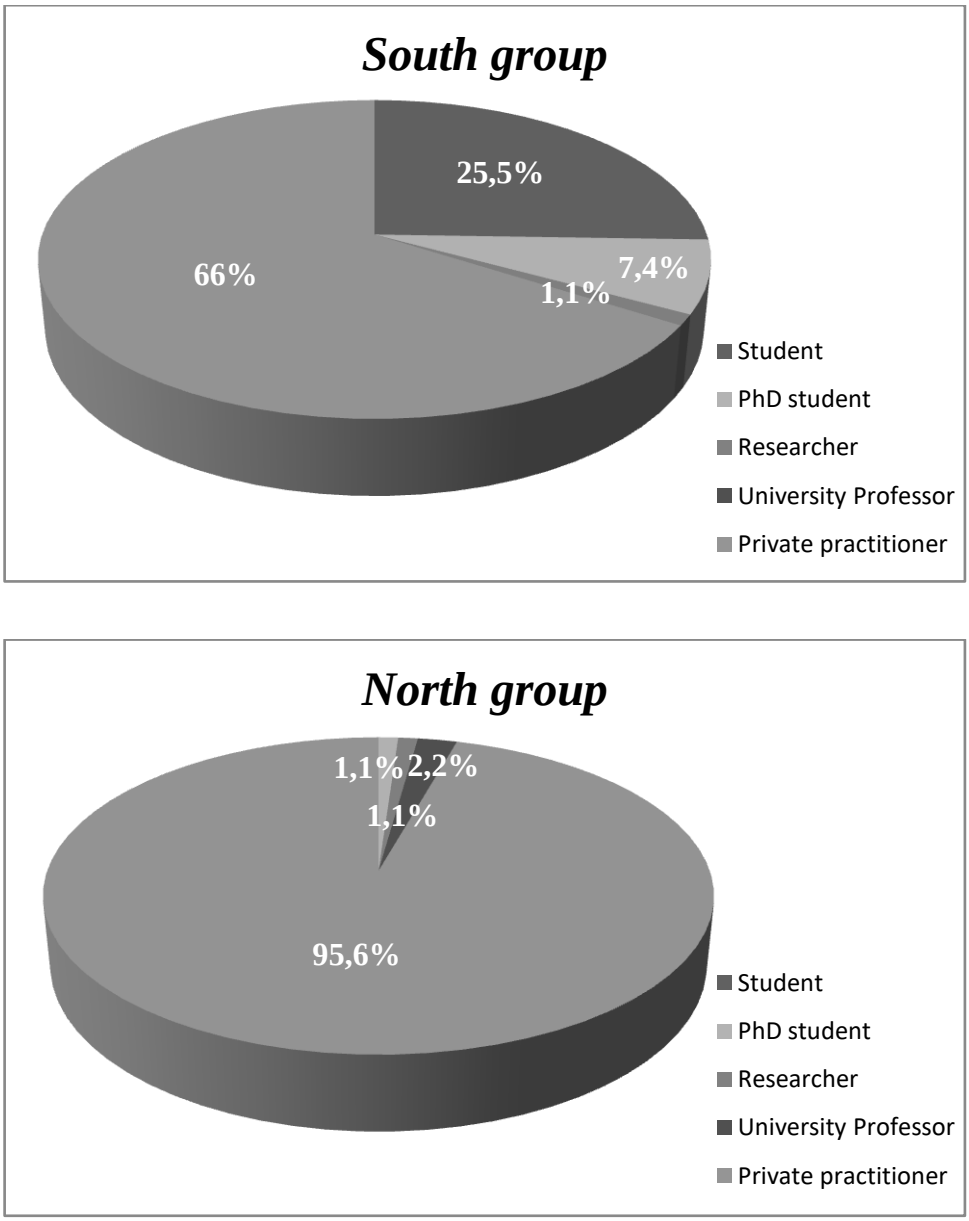


Figure 4. Professional levels (Student, PhD student, Researcher, University Professor and Private practitioner) of respondents in the South and North groups

Table 2. Provinces of professional activity of North and South groups respondents (number [n] and percentage [%])

South group			North group		
Province	***n***	***%***	***Province***	***n***	***%***
Messina	45	47.4	*Perugia*	24	26.7
Catania	10	10.5	*Milan*	9	10
Palermo	8	8.4	*Trieste*	1	1.1
Trapani	1	1	*Modena*	7	7.8
Enna	1	1	*Venice*	2	2.2
Syracuse	1	1	*Vercelli*	4	4.4
Ragusa	1	1	*Florence*	2	2.2
Agrigento	4	4.2	*Rome*	7	7.8
Reggio Calabria	2	2.1	*Pistoia*	1	1.1
Cosenza	8	8.4	*Lucca*	5	5.5
Lecce	8	8.4	*Ancona*	1	1.1
Macerata	2	2.1	*Teramo*	2	2.2
Campobasso	1	1	*Bologna*	4	4.4
Bari	1	1	*Genova*	1	1.1
			Latina	9	10
			Parma	1	1.1
			Verbania	1	1.1
			Verona	1	1.1
			Brescia	1	1.1
			Bergamo	1	1.1
			Padua	1	1.1
			Biella	5	5.5

Answers to the second section regarding pain assessment showed that most of the respondents assessed to judge their current knowledge on pain assessment and analgesia to be mild (52.2% in the South group and 61.8% in the North group). In the South group, 13% of respondents judges their knowledge to be very low, 23.9% advanced and 10.9 specialized, whereas in the North group the percentages were 6.7%, 25.8% and 5.6%, respectively. In both groups, most of the respondents assessed to use clinical evaluation to assess pain level (46.7% and 60.7% in the South and North groups, respectively). The most used pain scale was the canine Glasgow Composite Pain Scale (39.1% and 40.5% in the South and North group, respectively). Regarding the feline species, only 26% (South group) and 22.7% (North group) used a pain scale specific for cats, with the feline Glasgow Composite Pain Scale the most used (13% and 14.3% in South and North groups, respectively), whereas the UNESP-Botucatu was used only by the 4.3% and 3.6% of the respondents in the South and North groups, respectively. Answers to the third question of the second section showed that, almost all the parameters listed, are used to assess pain in dogs and cats (Table 3). Answers to the last questions of the second Section on the degree of pain assigned to different pathological and surgical conditions, are reported in Table 4.

Table 3. Most frequently used parameters to assess pain in dogs and cats. Number of citation (n) given for each parameter in both the South and North groups

Parameter	South group		North Group	
	Dog *(n)*	***Cat*** *(n)*	***Dog*** *(n)*	***Cat*** *(n)*
Behavioural changes	77	69	75	85
Vocalizations	72	50	72	42
Decreased appetite	79	71	65	76
Body tension	65	53	64	49
Aggression	66	66	60	63
Reaction to palpation	79	70	81	73
Heart rate	75	63	57	46
Respiratory rate	78	68	70	69
Arterial blood pressure	43	36	30	22
Decreased grooming	16	70	12	67
Reaction to stimulation	55	56	58	52

Table 4. Degrees of pain (mean ± Standard Deviation, SD) assigned to different pathological and surgical conditions by male and female respondents, in both the South and North groups

Condition	South group *(mean±SD)*		North group *(mean±SD)*	
	Male	***Female***	***Male***	***Female***
*ATE**	2.57±1.1	3.15±0.9	2.85±1.2	3.18±0.9
Bone fracture	3.18±0.7	3.20±0.8	3.34±0.5	3.16±0.6
*CNS** diseases*	2.26±1	2.21±0.9	2.34±0.9	2.42±0.9
Cystitis	1.92±0.8	1.98±0.7	2.35±0.5	2.29±0.6
Diaphragmatic hernia	2.20±1	2.39±0.9	2.43±1	2.46±0.7
*GDV***	3.13±0.9	3.08±0.8	3.09±0.6	3.22±0.7
Hepatitis	2.03±0.8	2.36±0.8	2.20±0.8	2.37±0.6
Neoplasia	2.44±1.1	2.38±1	2.40±1	2.59±0.8
Ocular diseases	2.40±0.8	2.22±0.9	2.56±0.8	2.71±0.7
Orthopaedic surgery	3.01±0.9	3±0.8	3.25±0.5	3.10±0.5
Osteoarthritis	2.62±0.8	2.69±0.7	2.68±0.5	2.87±0.5
Otitis	2.39±0.8	2.09±0.8	2.60±0.9	2.45±0.7
Ovariohysterectomy	1.93±0.8	2±0.6	2.16±0.8	2±0.6
Pancreatitis	3.26±0.8	3.23±0.8	3.28±0.8	3.40±0.6
Peritonitis	3.05±0.8	3.10±0.7	3.10±0.9	3.21±0.6
Pleuritis	2.44±0.7	2.64±0.8	2.62±0.8	2.73±0.6
Polytrauma	3.27±0.7	3.28±0.8	3.82±0.7	3.35±0.7
Pyelonephritis	2.39±0.9	2.61±0.9	2.63±0.9	2.87±0.7
Skin lacerated wounds	2.09±0.9	2.16±0.7	2.16±0.9	2.13±0.7
Spleen torsion	3.08±0.8	3.21±0.7	3.25±0.4	3.40±0.7
Urethral obstruction	2.90±1	3.16±0.8	3.35±0.6	3.26±0.6

*Arterial Thromboembolism

**Central Nervous System

***Gastric Dilatation Volvulus

In the South group, 6.5% of the respondents assessed to use analgesics only rarely (months), 21.5% sometimes (weeks), 38.7% often (days) and 32.3% every day, whereas in the North group, the percentages were 2.2%, 11.2%, 42.7% and 43.8%, respectively. Moreover, in the South group 1 person (1.1%) stated to never use analgesics. Among the reasons for a minimal or no use of analgesia, the most commonly chosen reasons in the South group were "wish to limit animal movement after a surgical procedure" and "fear of hiding the course of the disease" (27.3%), "lack of knowledge on analgesic pharmacology", "fear of side effects" and "cost of the analgesics" (22.7%), and "difficulty in pain assessment" (18.2%). In the North group, the most chosen reason for a low use of analgesics was "lack of knowledge on analgesic pharmacology" (50%), followed by "wish to limit animal movement after a surgical procedure" and "fear of side effects" (37.5%), "low attitude" (25%), "difficulty in pain assessment" and "cost of the analgesics" (12.5%).

The most used analgesic classes were, in both groups, NSAIDs (81.1% in the South group and 90% in the North group), followed by opioids (70% and 80%), local anaesthetics (31.1% and 31.1%), corticosteroids (26.7% and 32.2%) and α_2-agonists (27.8% and 35.6%).

The percentages of use of the different analgesics are reported in Table 5.

Table 5. Percentages (%) of use of the different analgesics in the South and North groups

Analgesic	***South group (%)***	***North group (%)***
Betamethasone	14.6	16.7
Buprenorphine	21.3	44.4
Butorphanol	37.1	57.8
Carprofen	38.2	50
Coxibs	40.4	44.4
Dexamethasone	30.3	28.9
Dexmedotomidine	34.8	36.7
Fentanil	39.3	50
Ketamine	23.6	35.6
Ketoprofen	19.1	21.1
Medetomidine	25.8	23.3
Meloxicam	69.7	80
Methadone	52.8	58.9
Methylprednisolone	20.2	15.6
Morphine	23.6	15.6
Tolfenamic acid	4.5	12.2
Tramadol	68.5	73.3

Almost all the respondents assessed to decide which analgesic to use based on each specific case and on the degree of pain perceived. In both groups, the most common reason for the choice of a specific class of analgesic was the personal experience and confidence with each specific active principle. Moreover, several respondents assessed that they usually prefer to administer NSAIDs and tramadol because of the bureaucratic restrictions related to the use of other analgesics, such as pure opioids and ketamine. Most of the respondents of both groups made use of the preventive analgesia (77.7% and 85.1% in the South and North groups, respectively).

Finally, most of the respondents (65.6%) of the North group assessed to be quite satisfied ("enough") of their degree of knowledge on pain therapy, while 4.4% answered "in no way", 26.7% "not much" and 3.3% "absolutely yes". In the South group, instead, 44.1% and 45.2% answered "not much" and "enough", respectively, 8.6% "in no way", and only 2.2% "absolutely yes". Among the most commonly used sources of information on pain management and analgesia, in the South group 66.3% answered "books", 64.1% "scientific articles" and 63% "seminars/congresses", while in the North group 48.9%, 71.1% and 67.7% answered "books", "scientific articles" and "seminars/congress", respectively.

Statistical analysis did not show any statistical significant difference between the North and South groups regarding the severity score assigned to the different pathological and surgical painful conditions with exception of bone fractures ($p= 0.02$) and pyelonephritis ($p= 0.03$). Regarding bone fractures, in age group 31-40 years, female veterinarians provided a higher score compared to male (Figure 5). In the North group most of the respondents (45.45%) described the degree of pain felt in case of bone fractures as severe, whereas in the South one the highest percentage (24.24%) chose terrible. Also regarding pyelonephritis, female veterinarians of the 41-50 years group provided higher score compared to male of the same age group. Moreover, the North group provided a higher score compare to the South one (Figure 6).

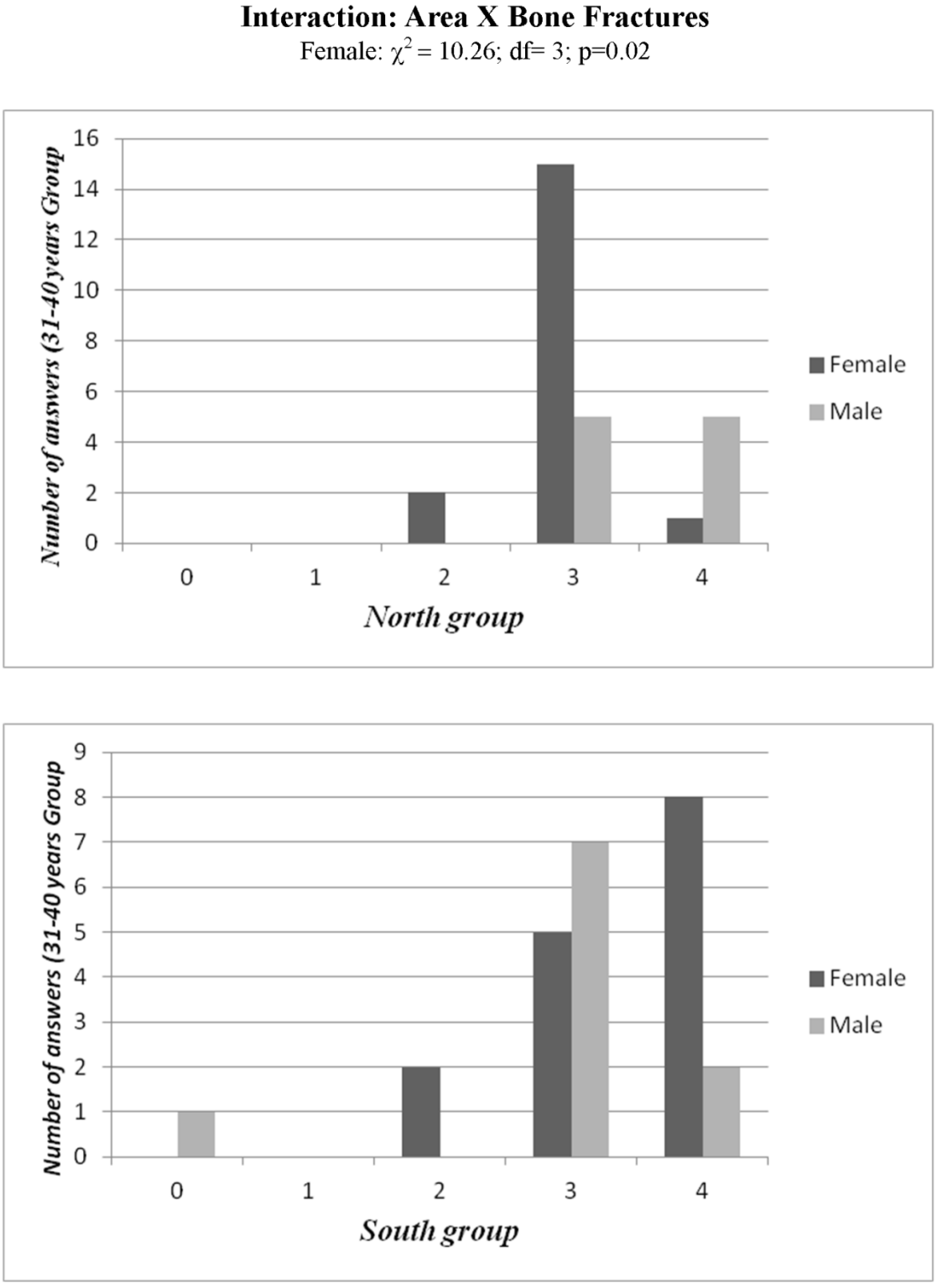


Figure 5. Comparison of the severity scores assigned to bone fractures between the South and North groups

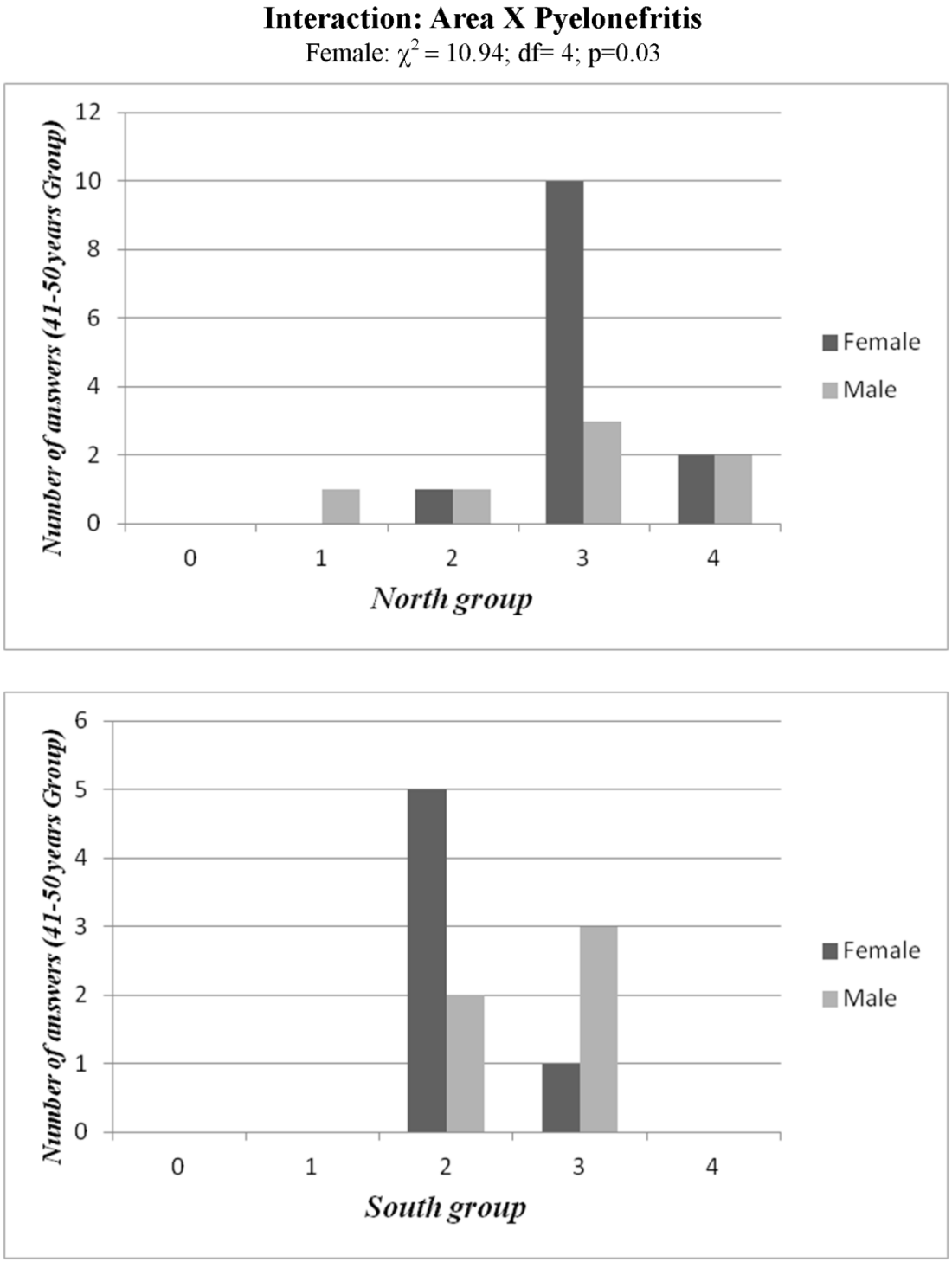
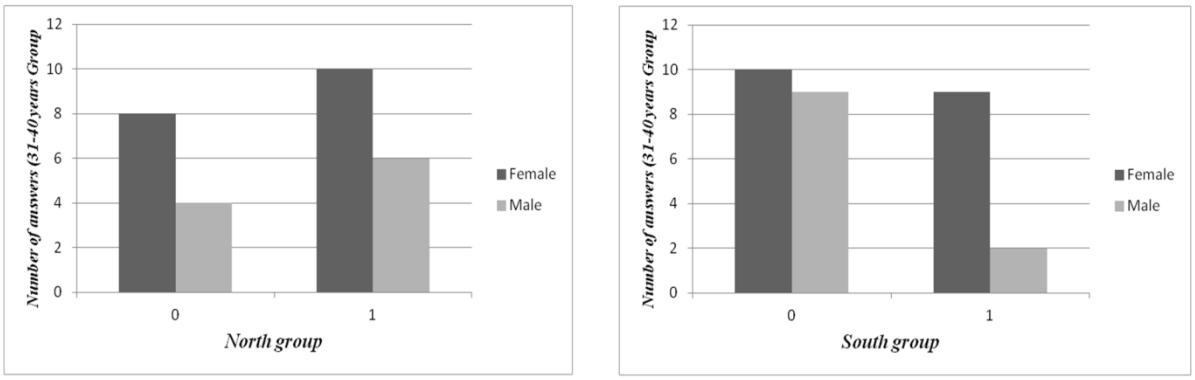


Figure 6. Comparison of the severity scores assigned to pyelonephritis between the South and North groups

Regarding the frequency of use of the different analgesics in the South and North groups, statistical analysis found statistical significant differences on the use of butorphanol, buprenorphine, carprofen, ketamine, meloxicam, methylprednisolone and local anaesthetics. In particular, butorphanol, carprofen, ketamine (Figure 7) and buprenorphine (Figure 8) were more used in the North group, whereas meloxicam, methylprednisolone and local anaesthetics (Figure 9) in the South one.

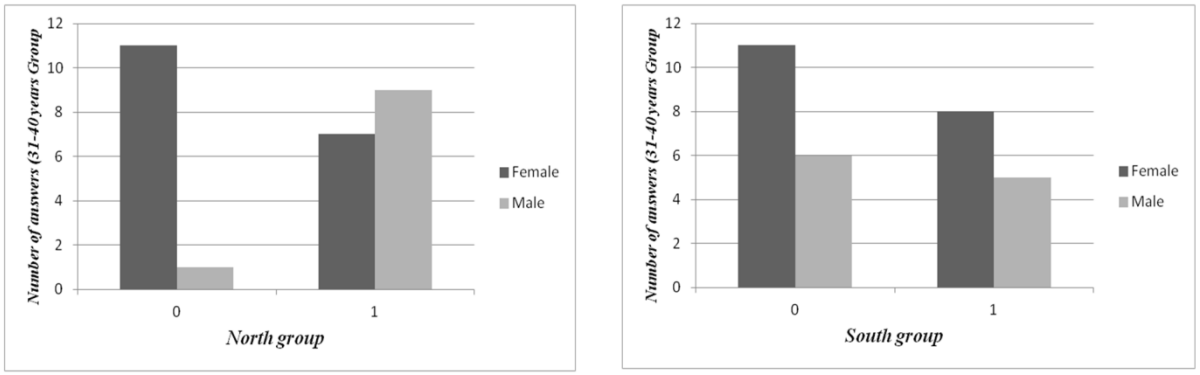
Interaction: Area X Butorphanol

Male: $\chi^2 = 3{,}88$; df= 1; p=0.048



Interaction: Area X Carprofen

Male: $\chi^2 = 4.68$; df= 1; p=0.03



Interaction: Area X Ketamine

Male: $\chi^2 = 3.88$; df= 1; p=0.048

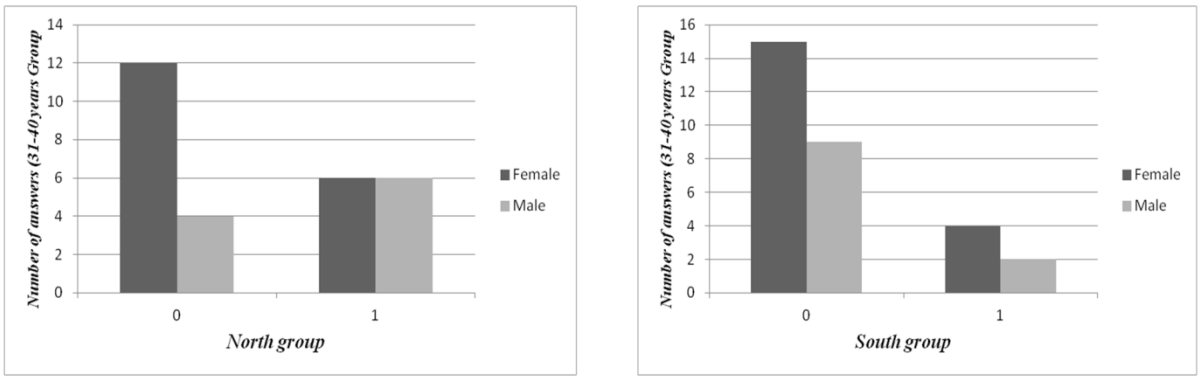
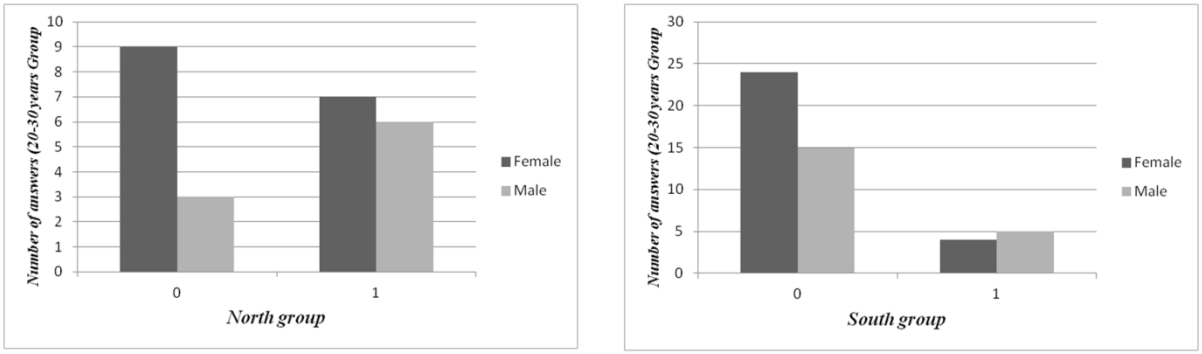


Figure 7. Comparison of the percentage of use of butorphanol, carprofen and ketamine between the South and North groups

Interaction: Area X Buprenorphine

Female: $\chi^2 = 4{,}72$; df= 1; p=0.03

Male: $\chi^2 = 4{,}58$; df= 1; p=0.03



Interaction: Area X Buprenorphine

Male: $\chi^2 = 4.20$; df= 1; p=0.04

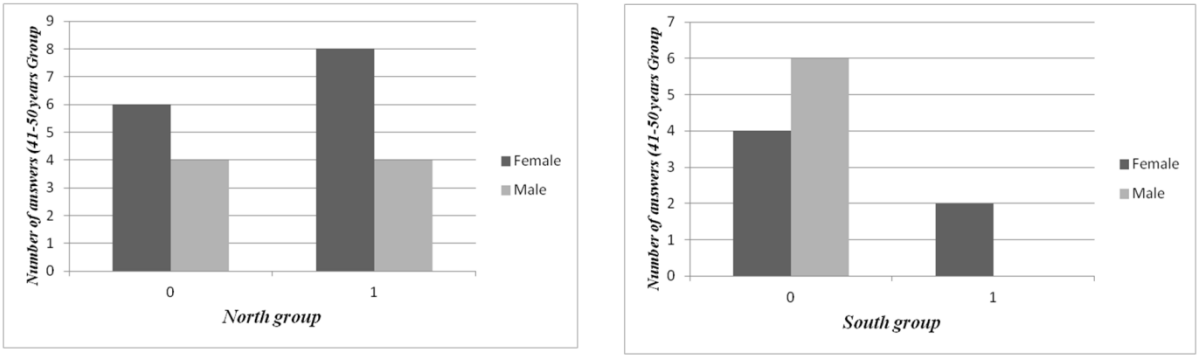
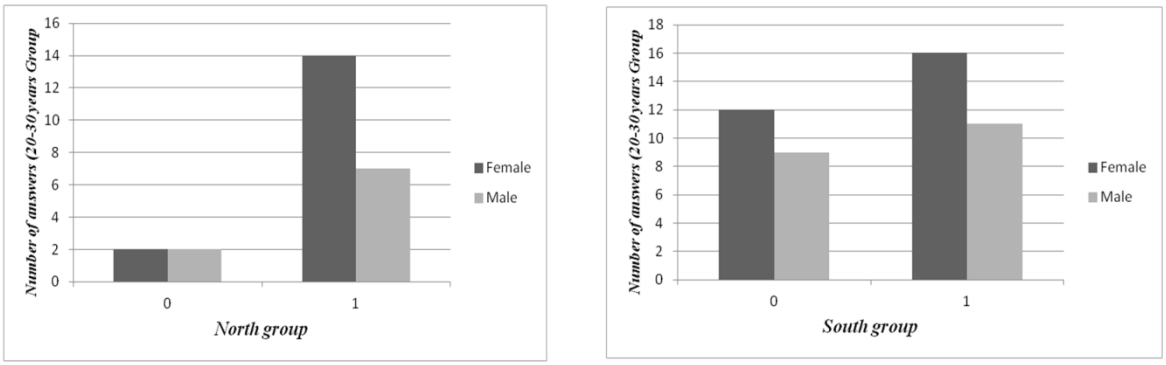


Figure 8. Comparison of the percentage of use of buprenorphine between the South and North groups

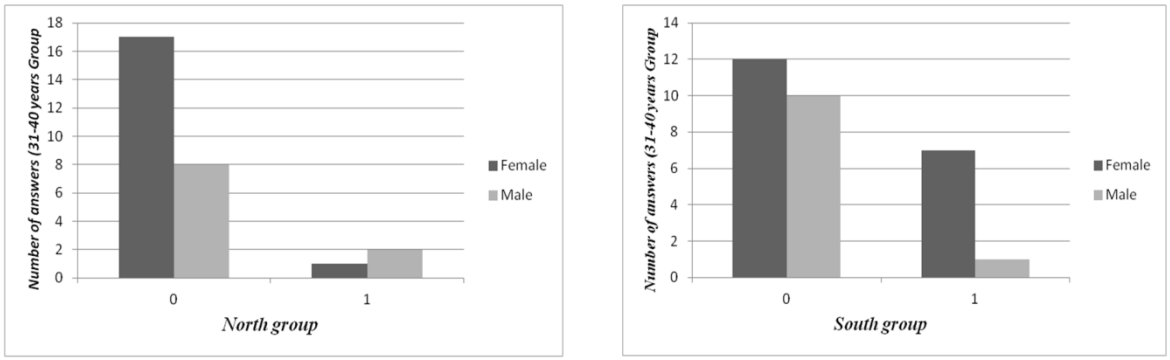
Interaction: Area X Meloxicam

Female: $\chi^2 = 4.33$; df= 1; p=0.04



Interaction: Area X Methil-prednisolone

Female: $\chi^2 = 5.34$; df= 1; p=0.02



Interaction: Area X Local Anaesthetic

Male: $\chi^2 = 5.18$; df= 1; p=0.02

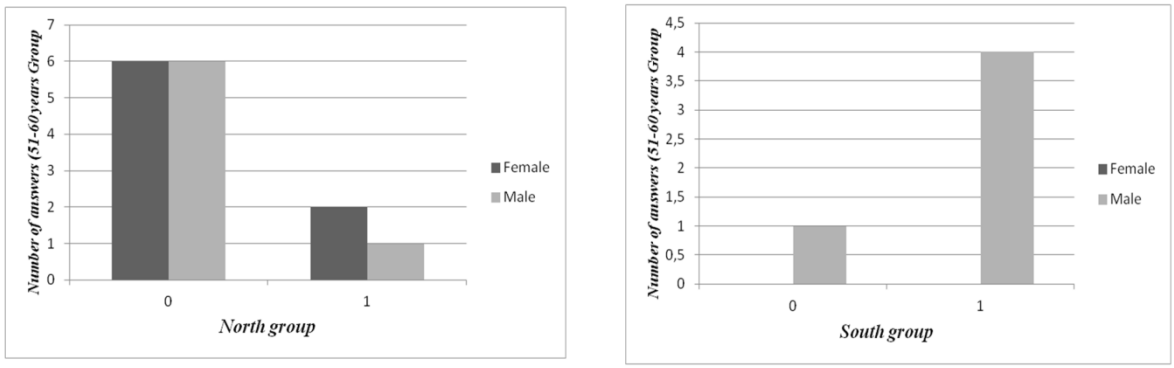


Figure 9. Comparison of the percentage of use of meloxicam, methylprednisolone and local anaesthetics between the South and North groups

4.1.4 Discussion

This survey aimed to investigate attitudes and knowledge toward pain assessment and analgesia among Italian veterinary practitioners and to evaluate if differences exist between veterinarians of the South and of the North of Italy. Considering that the survey was carried out using an online format, it was not possible to know how many people actually received the mail with the web link and, so, it was not possible to calculate the response rate. The response rate to this survey has been probably low, since the questionnaires has been sent to all the veterinary associations of each Italian province. However, it is not possible to know if the mail with the web link associated with the questionnaire has never been received by some associations, has never been readdressed to or filled in by the veterinary practitioners of those provinces. Moreover, even in the provinces where the veterinary associations answered to the presentation mail and readdressed the questionnaire to their veterinarians, the response rate was usually quite low. The low response rate could be the consequence of a poor interest in the topic of pain and analgesia or of a sub-optimal diffusion route of the questionnaire. Moreover, it has been shown that mailed survey had a lower response rate (67%) than face-to-face ones (Kelley et al., 2003). However, this way was chosen in order to try to reach the widest number of veterinarians, even that ones who lives far away from where the survey was developed.

The two provinces with the highest number of respondents were Messina, in the South group (45 respondents), and Perugia in the North one (24 respondents). This could be probably due to the fact that the questionnaire was developed at the University of Messina, so it was directly spread also among the students of the 4^{th} and 5^{th} years of the Veterinary Medicine course. The high response rate in the province of Perugia could be due to the fact that the Study Centre on Animal Pain (Centro di Studio sul Dolore Animale, CeSDA) is located at the Veterinary University of Perugia. Moreover, the authors of the only other survey on the topic in Italy, belong to these institutions.

As already reported in other studies (Catanzaro et al., 2016; Lorena et al., 2014; Hewson et al., 2006; Hugonnard et al., 2004; Raekallio et al., 2003), in both groups most of the respondents were female (55.9% and 61.4% in the South and North group, respectively), aged between 20 and 40 years old. This could be due to higher sensibility toward the topic of pain among women and young veterinarians. However, considering that the survey was based on an online questionnaire, the highest response rate among young could also be due to a greater skills with computer science compared to older people.

The decision to include also the students of the 4th and 5th years of Veterinary Medicine courses could have decreased the reliability of the answers, especially regarding pain scores attributed to pathological and surgical conditions, and to the types of analgesics used. However, since nowadays students of the last years of Veterinary courses are directly involved in the activity of every Veterinary Teaching Hospital, it was decided to address the survey also to this category. Moreover, students are the most direct and reliable representation of the University education.

Despite surgical and orthopaedic pathologies are among the most common painful conditions in veterinary medicine, the main area of interest of most of the respondents was internal medicine (63.7% and 59.6% in the South and North groups, respectively), while surgery was chosen only by 34.1% and 36% of respondents in the South and North group, respectively. Even lower was the percentage of respondents involved in reproduction (2.2% and 4.5%). This could be due to the fact that some veterinarians, especially surgeons, still consider a mild degree of pain as a "necessary tools" to ensure animal immobility, so reducing the risk of a damage of the surgical area due to excessive movements. In fact, 27.3% in the South group and 37.5% in the North one of the respondents describe the "wish to limit animal movement after a surgical procedure" as a reason for a reduced use of analgesics.

Despite the "difficulty in pain assessment" was another common reason for a reduced use of analgesics, most of the respondents (46.7% and 60.7% in the South and North group,

respectively) assessed to use “clinical evaluation” to assess pain, while only 39.1% and 40.5% used the Glasgow Composite Pain Scale, which is the only one validated for the dog. The percentage of use of pain scales was even lower for the cat, with only 4.3% and 3.0% using the UNESP-Botucatu and 13% and 14.3% the feline Glasgow Composite Pain Scale, in the South and North groups, respectively. Moreover, among the respondents, 5.4% and 2.4%, in the South and North group, respectively, assessed to not use any type of pain evaluation. These findings are in accordance with the ones of previous surveys (Catanzaro et al., 2016; Hugonnard et al., 2004). The development and use of validated pain scales for dogs and cats could be a good help in animal pain assessment. Moreover, the possibility to assign an objective pain score to each patient, could be very important in big veterinary practices where many veterinarians rotate during the different shifts. However, pain scales are often time-consuming and impractical, and most of the time the veterinary practitioner does not have enough formation on their use (Catanzaro et al., 2016). Finally, the lack of pain scales validated in the Italian language could explain the reduced clinical use in this country (Catanzaro et al., 2016).

Almost all the respondents assessed to use all the clinical parameters provided for assessing pain, with no relevant differences between dogs and cats. Only vocalizations and reduced grooming were more chosen for dogs and cats, respectively.

When it was asked to assign a degree of pain to different pathological and surgical conditions, the highest score were assigned to bone fracture, GDV, orthopaedic surgery, pancreatitis, peritonitis, polytrauma, spleen torsion and urethral obstruction, while the lowest to cystitis and ovariohysterectomy. Unlike previous studies (Catanzaro et al., 2016), it was decided to give verbal instead of numerical scores for pathological and surgical conditions because it was thought to be easier to assign an adjective than a number to a pain sensation. For statistical analysis, each adjective has been converted into a specific number (1 for slight, 2 for moderate, 3 for severe and 4 for terrible).

When it was asked the frequency of use of analgesics, most of the respondents in the North group chosen “often (days)” (42.7%) and “every day” (43.8%). Only 2.2% and 11.2% assessed to use analgesics rarely or sometimes, respectively. In the South group, instead, a higher percentage of respondents answered to use analgesics only sometimes (21.5%) compared to the North group, while 6.5%, 38.7% and 32.3% choose “rarely”, “often (days)” and “every day”, respectively. Moreover, one respondents in the South group assess to “never” use analgesics. However, considering the high percentage of students among the South group, this answer could have been provided by one of them.

Regarding the classes of analgesics, opioids and NSAIDs were the most commonly used. In particular, among NSAIDs meloxicam (69.7% and 80% in the South and North group, respectively) and coxibs (40.4% and 44.4%) were the most chosen. Unlike previous studies that reported that the most used opiod was butorphanol (Catanzaro et al., 2016; Hewson et al., 2006), the findings of this study showed that, among the respondents, the most commonly used opioid was methadone (52.8% and 58.9%). However, butorphanol was also very common in the South group (37.1%) and almost as used as methadone in the North group (57.8%). In this study, tramadol was the second most frequently cited analgesic (68.5% and 73.3% in the South and North groups, respectively) after the meloxicam. This high percentage of use could be due to the fact that in Italy, this drug does not require record-keeping or regulatory control, making it readily available to veterinary practitioners. Moreover, several clinical studies have shown that injectable tramadol provides a good postoperative analgesia in dogs undergoing ovariohysterectomy (Morgaz et al., 2013; Mastrocinque and Fantoni, 2003), mastectomy (Teixeira et al., 2013) and hemilaminectomy for Acute thoracolumbar intervertebral disk extrusion (Giudice et al., 2017). Local anaesthetics were used in both groups by the 31.1% of respondents, in accordance with the results reported in previous study (Hugonnard et al., 2004; Perret-Gentil et al., 2014). Loco-regional procedures are simple to perform, carry low risk, are not expensive and do not require the special prescription for

opioids, so representing a good alternative for pain management. Therefore, the relatively low rate of use of these analgesics is probably related to a lack of knowledge on the loco-regional techniques, that should be more studied by veterinary practitioners. Corticosteroids were used for the analgesia by the 26.7% and 32.2% of the respondents in the South and North groups, respectively. Among them, dexamethasone was the most commonly used (30.3% and 28.9%, respectively). Although the percentage of use was lower than in previous studies (Hugonnard et al., 2004), the findings of this survey showed that corticosteroids are still widely used to treat pain, despite the several side effects they could determine.

Statistical analysis showed that the responders of the South group used more meloxicam, methylprednisolone and local anaesthetics compared to the North one. On the contrary, the percentage of use of butorphanol, buprenorphine and ketamine was significantly higher in the latter. This could be due to the fact that in the South, veterinary small clinical practices are more common than big clinics and hospitals. In the formers, due to the small number of patients, it could be economically not convenient to hold a register for narcotic drugs, so determining a greater use of NSAIDs, corticosteroids and local anaesthetics.

Finally, when it was asked to the respondents how they judged their level of knowledge on pain therapy, most of the veterinarians of both groups answered “not much” and “enough”. This could be due to the fact that, nowadays, more veterinarians than in the past are aware of the importance of pain management and of the difficulty of pain assessment, so understanding that a constant formation is primary in order to be able to properly diagnose and treat a painful condition.

Although it is possible that, by giving multiple choice questions, the answers could have been influenced by the ones proposed in the lists, this choice was made in order to simplify, as much as possible, the compilation of the questionnaire, so that it could have been easily and quickly filled in. On the contrary, open questions can be time consuming for the responders and difficult to analyze (Kelley et al., 2003).

Advantages of surveys are that the data collected are based on a real-world observation and that, by covering a big number of people, the data collected could be generalized to the whole population considered. Disadvantages are the difficulty of achieving a high response rate, that the data collected are likely to lack details on the topic investigated and that the significance of the data can be neglected if the researcher focus too much on the coverage range, ignoring the weight of these data on the issue investigated (Kelley et al., 2003).

To summarize, the findings of this survey show that most of the respondents in both groups assessed to have mild or advanced knowledge on pain assessment and analgesia. However, most of the veterinarians assess pain severity only through clinical evaluation, without using pain scales. Among the most commonly analgesics used, NSAIDs and opioids were the most commonly cited. However, still a moderate number of veterinarians use butorphanol and corticosteroids to treat pain. Finally, no statistical differences were found in the degree of pain assigned to different pathological and surgical conditions and in the use of the different analgesics between the South and the North groups.

In conclusions, although the knowledge and attitude of Italian veterinarians are improving and can be compared with the ones of other veterinarians all around the world, a better formation should be provided by the University and post-graduate courses and more attention in general should be given to the topic of pain and analgesia.

4.2 Pain management in dogs with acute thoracolumbar intervertebral disc extrusion and degenerative lumbosacral stenosis: comparison of different analgesic protocols

Neuropathic pain

The International Association for the Study of Pain (IASP) define neuropathic pain as a "pain initiated or caused by a primary lesion or dysfunction in the nervous system" (Merskey e Bogduk, 1994). To clarify the unspecific term of "dysfunction", another definition described neuropathic pain as a "pain that develops as a direct result of diseases that affect the somatosensory system" (Treede et al., 2008). In human medicine, neuropathic pain has been reported to occur in 1% to 3% of patients seen in pain clinics (Hayes et al., 2002). A study in veterinary medicine, instead, has reported a higher prevalence of neuropathic pain in dogs (8%) and cats (7%) (Muir et al., 2004).

The algia results from a continuous or permanent damage of the peripheral or central nervous tissue, such as damage of the somatosensory and visceral peripheral nerves, meninges, spinal cord and its nerve roots, or lesion to the brain. Neurons are protoplasmatic extensions of live cells, and damages to the axon or soma (like dysmyelination and demyelination) directly confers pain (Niv and Devor, 2006). Neuropathic pain does not provide any benefit to the organism and can be considered as a disease in itself. Although it is frequently associated with chronic diseases, it can also develop as a result of acute conditions, such as trauma involving the nervous tissue or surgery that may have resulted in iatrogenic injury (Treede et al., 2008). Moreover, also poorly managed acute or chronic pains can result in neuropathic pain. In fact, severe noxious and inflammatory stimuli are mediated by the same pathways, resulting in changes within the nervous system (Taylor, 2001).

Neuropathic pain results from an abnormal activation of neuronal pathways responsible of pain perception, which arises from damage or dysfunction of the peripheral nerves and their dorsal root (peripheral neuropathic pain), or of the spinal cord and brain (central neuropathic pain). The main two processes that rouse neuropathic pain are abnormal peripheral input and abnormal central processing. Unlike the inflammatory one, neuropathic pain occurs in absence of nociceptors stimulation, but for ectopic activation of peripheral neurons and long-term changes in nociceptors, ascending fibres, thalamic and cortical neurons. For this reason, it is considered a disnociceptive pain. Although nociceptive processing is similar in human beings and domestic animals, the anatomic differences among species in the ascending fibres of the spinothalamic tract can probably determine a different and less defined characterization and localization ability, that may result in an increased awareness of the adverse quality of a stimulus and in increased autonomic responses in animals (Mathews, 2008). Ectopic firings of damaged neurons tend to occur and the quality of the pain sensation differs from nociceptive or inflammatory pain. This difference seems to be related to a reorganization of transmission pathways within the nervous system after nerves injury (especially changes in the Aβ-fibres), such as alterations in the expression of neurotransmitters, neuromodulators, receptors (substance P receptors) and ion-channels. Moreover, Aβ-fibres can activate C-fibres, so that also innocuous stimuli applied to the area innerved by the damaged nerveare interpreted centrally as a noxious stimulus (allodynia). Finally, during the healing process, Aβ-fibres may establish connections with C-fibres, perpetuating the allodynia phenomenon (Matthews, 2008). Another process that has been identified to be involved in the development of neuropathic pain in humans is called “noradrenergic supersensitivity”. Normally, primary afferent nerve endings are not sensitive to catecholamines and sympathetic activity does not cause pain. In case of nerve damage, instead, release of norepinephrine increases the electric discharge of unmyelinated fibres. Clinical signs that may lead to the suspect of a sympathetic contribution to neuropathic pain is the concomitant presence of skin vasomotor activity,

sweating, and worsening of pain due to stimuli that evoke a sympathetic response (McLachlan et al., 1993). Inflammatory and immune mechanisms within the peripheral and central nervous system play also an important role in neuropathic pain. Infiltration of inflammatory cells and activation of immune cells lead to the production of various inflammatory mediators (especially Tumour Necrosis Factor-α, TNF-α, and interleukin-1β, IL-1β) which can increase afferent neurons sensitization and contribute to hypersensitivity (central sensitization) (Moalem and Tracey, 2006). Finally, various studies has demonstrated a lower efficacy of descending inhibitory pathways, especially reduce opioid receptors function, in animals with neuropathic pain (central disinhibition) (Mayer et al., 1995). Decreased inhibition at the level of the dorsal horn of the spinal cord results in a higher likelihood of neurons firing spontaneously or more energetically to afferent input (Mathews, 2008). Norepinephrine and serotonin are two of the major components of the endogenous descending inhibitory system and a reduction in their levels at the spinal and supraspinal levels may be involved in chronic pain. It seems that norepinephrine and serotonin reuptake inhibitors (SRIs) may attenuate pain by determining increased postsynaptic levels of the two mediators (Ren and Ruda, 2002).

If the diagnosis of nociceptive, physiological pain, is difficult in non verbal veterinary patients, the diagnosis of neuropathic pain can be extremely difficult, unless a predisposing lesion or injury is identified. The presence of signs such as hyperalgesia (exaggerated response to painful stimuli) and allodynia (pain in response to normally innocuous stimulus), in combination with deviations of the usual behaviour of the animal and in the absence of an obvious cause, can provide the suspect of neuropathic pain. Neuropathic pain should be considered also when an “unexpected” or “greater than expected” level of pain occurs after a surgery or trauma. When assessing pain in animals, it is also important to determine if pain is caused or increased by movement (movement-evoked pain) or pressure (e.g., pressing around surgical wounds).

Some conditions are well-known to cause neuropathic pain in dogs and cats, and can be associated with surgery (inguinal hernia repair, pelvic fractures, pudendal or limb nerves entrapment, and limb amputation), trauma (lumbosacral lesions, spinal cord injury, intervertebral disc extrusion or herniation), fibrocartilaginous embolic myelopathy, discospondylitis and vertebral osteomyelitis, polyradiculoneuritis, diabetic neuropathy, tumours of the central nervous system, congenital abnormalities and vasculitis. Moreover, neuropathic pain may have a visceral origin, like in case of feline interstitial cystitis, inflammatory bowel disease and pancreatitis.

Acute thoracolumbar intervertebral disc extrusion

Acute thoracolumbar intervertebral disc extrusion is a common cause of neuropathic pain in dogs and, less frequently, in cats. It is characterized by a chondroid degeneration of the nucleus pulposus. It starts around 6-8 months of age, especially in chondrodystrophic breeds (e.g., Dachshund, Pekinese, Shih tzu, Beagle, Cocker spaniel), but it has also been described in medium-large breeds (e.g., Sheepdogs) (Cudia and Duval, 1997). The degree of pain experienced during acute disc extrusion is usually between severe and terrible (Carroll and Martin, 2007). At first, the degeneration is asymptomatic until the nucleus pulposus, flooded with hyaline cartilage, protrudes through a slot of the annulus fibrosus. Clinical signs and pain are caused by the compression of the meninges and nerve roots, but they are actually the final representation of a slow disc degeneration, which started months or, more often, many years before. It is still debated whether pain is caused also by the receptor stimulation in the annulus fibrosus and in the dorsal longitudinal ligament. Clinical signs depend on the amount of herniated material and the ratio between the diameter of the medulla and the vertebral canal. As a result of the initial mechanical event and persistent compression of the spinal cord, a cascade of secondary injury mechanisms, which exacerbate the degree of tissue destruction, occurs. These processes include free radical formation, cellular ionic imbalance, cell membrane lipid peroxidation, release of excitotoxic glutamate, and vascular phenomena, such

as vasospasm and perfusion-reperfusion injury (Wilson and Fehlings, 2011). The onset of clinical signs is generally acute, after a jump or a sudden movement while the dog is running or playing. Clinical manifestations begin at the age of 2 to 6 years, and the most common site of injury is the thoracolumbar region, especially from T11 to L3. The most common clinical signs are hyperesthesia, paresis, kyphosis, back pain and reluctance to walk, which are obvious consequences of pain (Coates, 2000).

Clinical diagnosis is based on the history and clinical data collected during the general physical and neurologic examinations. The final diagnosis is achieved by myelography and advanced diagnostic imaging. Computed tomography (CT) allows observation of the section of the medullary parenchyma in order to detect even lateral compressions and early parenchymal abnormalities, like myelomalacia. Magnetic resonance imaging (MRI) allows a comprehensive view of the spine, with a high definition display of the soft tissues (Cooper et al., 2014).

The therapy of choice is hemilaminectomy surgery, performed as early as possible to prevent the onset of irreversible spinal cord damage (Tator and Fehlings, 2010). Surgery has the goal of decompressing the spinal cord and restoring spinal stability. Although a consensus regarding the optimal timing of surgical decompression has not been reached yet, most of the preclinical and clinical evidences in human and animal models support performing early surgery (within 24 hours from the onset of clinical signs) (Wilson and Fehlings, 2011). However, beside the surgical treatment, post-operative pain management is extremely important in order to avoid continue acute pain that could develop into a chronic neuropathic pain.

Degenerative Lumbosacral Stenosis (DLSS)

Another common cause of neuropathic pain in dogs is the Degenerative Lumbosacral Stenosis (DLSS), also called *cauda equina* syndrome. The syndrome occurs mainly in large breed dogs, especially German Shepherd and working dogs, with a prevalence rate increasing with

age (mean age at presentation 7 years) and with male dogs more affected than female (Suwankong et al., 2008; De Risio et al., 2001; Danielsson and Sjöstrom, 1999). Individual and breed-related conformational problems may predispose the German Shepherd dog to the development of DLSS, whereas male may be more affected because they generally are heavier and grow faster than female dogs, which may predispose them to skeletal problems. (Danielsson and Sjöstrom, 1999). Dogs undergoing intensive work or physical activity and overweight are at a higher risk. Other breeds reported with DLSS are the Labrador Retriever, Golden Retriever, Border Collie, Dalmatian, Weimaraner, Belgian Shepherd, Schnauzer, Great Dane, Rottweiler, Newfoundland, Bernese Mountain Dog, German Longhaired Pointer, Bearded Collie, Leonberger, American Staffordshire Terrier, Dutch Partridge dog, and mixed-breed dogs (De Decker et al., 2014; Suwankong et al., 2008; Suwankong et al., 2006).

DLSS is caused by several degenerative alterations of the bone and soft tissues surrounding the *cauda equina* (from L7 to S1), resulting in the compression of the terminal part of the medulla. The "*cauda equina*" is composed by the ventral and dorsal roots of the spinal nerves L6, L7, S1-S3, and Cd1-Cd5 and it is surrounded by the L7-S1 vertebral bodies. During the embryogenesis, the spinal cord and the vertebral column develop at two different rates, resulting in a spinal column that is longer that the spinal cord (Meij and Bergknut, 2010). Several mechanisms take part in the pathophysiology of DLSS: Hansen type II (or, less commonly, type I) Intervertebral Disc (IVD) herniation, ventral subluxation of S1 (lumbosacral instability), congenital vertebral anomalies (such as transitional or extra vertebrae) (Fluckiger et al., 2006), proliferation of the soft tissues surrounding the cauda equina (e.g., hypertrophy of ligaments, the joint capsule, and epidural fibrosis), sacral osteochondrosis (Mathis et al., 2009) and vascular abnormalities compromising blood supply to the spinal nerves (Adams and Roughley, 2006). Whatever the underlying mechanisms is, all these alterations cause to the compression of spinal roots, leading to the development of clinical signs (Meij and Bergknut, 2010), even if they can also be due to alterations of other

adjacent anatomical structures. The compression causes the demyelization and inflammation of nerve roots with the release of various pro-inflammatory cytokine (TNF, IL-6 and IL-β) and growth factors, and the activation of astrocytes and microglia.

The most common clinical signs reported in dogs with DLSS are caudal lumbar or lumbosacral pain, pelvic limb lameness, hyperesthesia or self-mutilation of the lumbosacral area or pelvic limbs, difficulty with rising, sitting, or lying down, reluctance to jump or climb, dragging of toes, a low carriage of the tail, and urinary and fecal incontinence (Meij and Bergknut, 2010). Clinical signs can be acute or chronic, continuous or intermittent. Lumbosacral pain is caused by the compression of neural and vascular structures and is easily evoked by tail hyperextension, lumbosacral joint digital pressure and hind limbs hyperextension. Micro-vascularisation of nerve roots is relatively shallow when compared with that of the peripheral nerves and, therefore, more sensitive to compressive phenomena. In some dogs, nonweight-bearing pelvic limb lameness is the only clinical sign, caused by the unilateral compression of the L7 and/or S1 nerves with radiating nerve root pain. Most of the times, neurological signs are uncommon, because the *cauda equina* is resistant to compressive forces, and proprioception is usually normal. However, lower motor neuron signs, such as paresis, muscle atrophy, reduction in the withdrawal and increased in the patellar reflexes are commonly observed (Meij and Bergknut, 2010).

Diagnosis is initially based on history and neurological examination. Radiographs of the lumbosacral spine and myelography can show narrowing of the IVD space, sclerosis of vertebral end plates, subluxation of S1, ventral spondilosis, sacral osteochondrosis, vacuum phenomena, transitional or additional vertebrae or congenital sacral abnormalities (Steffen et al., 2007). Moreover, they can help to exclude bone neoplasia and traumatic luxation (Suwankong et al., 2006). However, normal radiographs are not enough to rule out a suspicion of DLSS. For this reason, more advanced techniques, such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI) and CT myelography, are essential to

achieve the diagnosis of DLSS. Several studies have reported a good correspondence of CT and MRI with surgical findings (Mayhew et al., 2002; Jones et al., 2000). However, other studies have reported a good correspondence between CT and MRI findings, but only a slight one between diagnostic imaging and surgical findings (Suwankong et al., 2006). Some studies have reported positive bacterial cultures (*Bacillus* spp., *Staphilococcus intermedius*) in swabs from disc material collected during surgery, even in dogs with no signs of discospondylitis on diagnostic imaging (Suwankong et al., 2008).

Dorsal laminectomy is the main surgical treatment performed in dogs with DLSS (Suwankong et al., 2008; Danielsson and Sjöstrom, 1999), with the aim of decompressing the *cauda equina* and free the entrapped nerve roots (Meij and Bergknut, 2010). If further decompression is required, the laminectomy may be extended laterally to include unilateral or bilateral facetectomy/foramenectomy when stenosis of the intervertebral foramina is present. Fenestration, nucleotomy, foraminotomy and fixation of S1 subluxation can be associated. The success rate reported after surgery ranged between 70% to 93% (Suwankong et al., 2008; De Risio et al., 2001; Danielsson and Sjöstrom, 1999). Factors that came out to be negative predictors of the outcome after surgery were the presence of urinary incontinence (Suwankong et al., 2008; De Risio et al., 2001) and the combination of laminectomy with other surgical procedures. This may be related to a higher degree of disc degeneration, requiring a wider surgery, or to a greater spinal instability following surgery that exacerbated the degenerative process (Suwankong et al., 2008). Although the prognosis for dogs surgically treated for DLSS is generally good, return to normal function is more likely in the least affected dogs, whereas severely affected dogs often show persisting neurologic deficits. Moreover, recurrence of clinical signs may occur after the surgical treatment.

Medical conservative treatment of DLSS usually consists of the administration of NSAIDs or corticosteroid, together with reduction of body weight and of physical activity (Meij and Bergknut, 2010). Physiotherapy, regular walks and underwater treadmill may help recovery

and improving muscle tone. Lumbosacral injections of corticosteroid have been shown to be an effective treatment in dogs with no proprioceptive deficits and urinary and/or fecal incontinence, showing an improvement in the 94% of the patients (Janssens et al., 2009). Medical treatment aims at controlling pain and does not solve the underlying problem. Although medical treatment has been proposed as an alternative to surgery, only one retrospective study exist on conservative treatment in dogs with DLSS (De Decker et al., 2014). In this retrospective study, dogs were treated with NSAIDs, gabapentin or a combination of both, with the 55% of dogs that underwent medical treatment were considered to have a successful outcome, based on clinical signs and owner's opinions.

In human medicine, several drugs have been used to treat lumbosacral stenosis and neuropathic pain in general. In human patients with lumbar stenosis, medical management have been reported to decrease back pain, but not leg pain, physical ability and quality of life (Parker et al., 2014). Among the drugs considered for the medical management of lumbosacral stenosis, gabapentin has been reported to provide a longer walking distance, lower pain score and better recovery of sensory deficit compared to standard treatment with NSAIDs and physiotherapy (Yaksi et al., 2007).

Buprenorphine

Buprenorphine, a partial μ-opioid receptor agonist, has a very high affinity with this type of receptors, which leads to the formation of a virtually unbreakable bond. It showed good analgesic properties, and this makes it a good choice for alleviation of mild to moderate pain (Slingsby et al., 2006; Shih et al., 2008). It has a fairly long-lasting action (6-8 hours) (Andaluz et al., 2009), and the maximum effect is reached about 20 minutes after intramuscular (IM) administration. Previous studies showed a decreased incidence of side effects, such as respiratory depression (Dahan et al., 2005). Recent evidences suggest that the submaximal response to buprenorphine at high doses may be because of the interaction with

the non-μclass of opioid receptor-like (ORL1) receptors, that, when upregulated, may attenuate the analgesic effects of buprenorphine (Lutfy et al., 2003; Lufty and Cowan, 2004).

Tramadol

Tramadol is a synthetic codeine analogue which provides pain relief through different levels of action, especially the inhibition of norepinephrine and serotonin uptake. Its primary metabolite, O-desmethyl-tramadol, shows an affinity with μ-receptors 20 to 200 times higher than tramadol itself (Kukanich and Papich, 2004). Especially the inhibition of the reuptake of norepinephrine and serotonin, is thought to contribute significantly to the drug's analgesic activity, possibly representing an advantages in humans and animals with neuropathic pain. Compared with other opioids, it appears to cause less respiratory depression and not cause problems of tolerance and abuse, except in a very limited way. It has a half-life of about 4-6 hours, and the onset of the analgesic effect is achieved after 10-20 minutes. Administered orally and parenterally in humans and animals, it seems to have a good analgesic activity on different types of pain (Perez-Jimenez et al., 2016). Side effects include dysphoria and sedation, especially in cats, and reduced seizure threshold in humans. Tramadol is often used in dogs for postoperative pain control because of its non-addictive effect and relatively low cost. In the United States, tramadol is classified as a narcotic drug, requiring a narcotic type prescription; however, in other countries, it is still considered a non-narcotic drug, so representing a good compound for short-term treatment at home.

Some studies have evaluated the analgesic effects of tramadol and buprenorphine in dogs undergoing ovariohysterectomy (Shih et al., 2008; Moll et al., 2011; Morgaz et al., 2013) or other surgical procedures (Bosmans et al., 2007; Slingsby et al., 2011; Linton et al., 2012), but there is a lack of report on neuropathic pain after spinal surgery. However, in human medicine tramadol has been used to treat neuropathic pain in patients with post-herpetic neuralgia and diabetic neuropathy, showing a good analgesic activity (Bennett, 2007b). However, the last Cochrane report (Duehmke et al., 2017) on the use of tramadol for neuropathic pain in human

adults assessed that, despite scientific evidences exist on the analgesic activity of tramadol against neuropathic pain, these evidences were of low quality, due mainly to the small numbers of patients enrolled in the clinical trials. This means that the researches do not provide a reliable indication and that new, larger trials are needed to know if tramadol is really useful for the management of neuropathic pain.

Gabapentin

Gabapentin (1-(aminomethyl) cyclohexane acetic acid) is an antiepileptic drug which is a structural analogous of γ-aminobutyric acid (GABA). However, it has been demonstrated that gabapentin does not bind to GABA-A or GABA-B receptors (Jensen et al., 2002), is not metabolically converted into GABA or a GABA agonist, and it is not a reuptake inhibitor of GABA (Cheng and Chiou, 2006). Several studies have shown that gabapentin blocks the channels of Ca^{2+} and Na^{+}, by binding the α_2-δ subunit of the voltage gated calcium channels (Taylor, 2009), and opens the one of K^{+}, acting presinaptically by decreasing the glutamate and glutaminergic synaptic transmission (Bertrand et al., 2001) and substance P release. Moreover, it has been described that gabapentin interacts with N-methyl-D-aspartate receptors (NMDA) involved in the development of central sensitisation (Pozzi et al., 2006), and activates the descending noradrenergic system and induces spinal norepinephrine release (Takeuchi et al., 2007). Gabapentin is mainly absorbed in the upper gastrointestinal tract in dogs (Stevenson et al., 1997), it appears in the plasma 30 minutes after oral administration in healthy Beagle dogs (Rhee et al., 2008) and is eliminated mainly by renal excretion. The half-life is reported to be about 5-7 hours. However, pharmacokinetic studies in healthy dogs and cats have shown a terminal half-life of 3.3 hours (KuKanich and Cohen, 2011) and 177±25 minutes (Siao et al., 2010), respectively. The efficacy of gabapentin in human is associated with 2μg/mL plasma concentrations, but the corresponding value in dogs has not been established. In the study of Kukanich and Cohen (2011), gabapentin exceeded 2 μg/mL in dogs that received a 10 mg/kg dose in 6/6 dogs at 6 h, 4/6 dogs at 8 h and 0/6 dogs at 12 h

after administration, suggesting that a dose of 10 mg/kg every 8 h should be enough to maintain 2 μg/mL plasma concentrations in dogs.

More recently, gabapentin has been found to be effective for the management of chronic and neuropathic pain. In particular, gabapentin was shown to be effective in reducing pain scores in human patients with diabetic neuropathy (Alvarado and Navarro, 2016) and lumbosacral pain (Yaksi et al., 2007). Moreover, gabapentin provided a better analgesia in women with chronic pelvic pain, compared with a placebo group (Lewis et al., 2016). Gabapentin analgesic mechanism is still not completely understood, but it showed anti-hyperalgesic and anti-allodynic effects in a rat model of induced neuropathic pain (Mangaiarkkarasi et al., 2015). In the veterinary literature, gabapentin has been used to treat pain both in dogs and cats. In a study in Cavalier King Charles Spaniel with neuropathic pain caused by Chiari-like malformation and syringomyelia, gabapentin analgesic activity has been compared with topiramate, another antiepileptic drug, administered as a supplemental treatment to carprofen. No statistical significant differences have been found in VAS scores between the two groups, but the gabapentin group showed a better quality of life compared to baseline and to topiramate group (Plessas et al., 2015). Gabapentin has also been used in dogs for postoperative treatment after intervertebral disc surgery (Aghighi et al., 2012), forelimb amputation (Wagner et al., 2010) and mastectomy (Crociolli et al., 2015), without any significant difference in pain scores compared to placebo groups, even if gabapentin group requires less morphine administration after mastectomy and showed lower pain score after intervertebral disc surgery. Only two reports exist on the use of gabapentin in cats suffering from multiple skull fractures and chronic forelimb lameness (Lorenz et al., 2012) and road traffic accident (Vettorato and Corletto, 2011). No significant adverse effects have been reported both in human and animal studies after treatments with gabapentin.

4.2.1 Aim of the studies

This chapter describes two clinical studies on neuropathic pain management in dogs with acute thoracolumbar intervertebral disc extrusion and degenerative lumbosacral stenosis (DLSS), respectively. Only a few reports exist in the veterinary literature on pain management in dogs affected by these two disease (De Decker et al., 2014), and no one has evaluated the analgesic activity of tramadol and buprenorphine and tramadol and gabapentin in acute thoracolumbar intervertebral disc extrusion and DLSS, respectively.

The aim of the first study was to compare the analgesic activity of tramadol and buprenorphine as postoperative pain management in dogs undergoing hemilaminectomy surgery because of acute thoracolumbar intervertebral disc extrusion. The aim of the second study was to report a medical management with tramadol and to describe the possibility of using gabapentin as an alternative treatment for lumbosacral pain in dogs affected by Degenerative Lumbosacral Stenosis (DLSS).

4.2.2 Materials and methods

Comparison of the analgesic activity of buprenorphine and tramadol in dogs undergoing hemilaminectomy

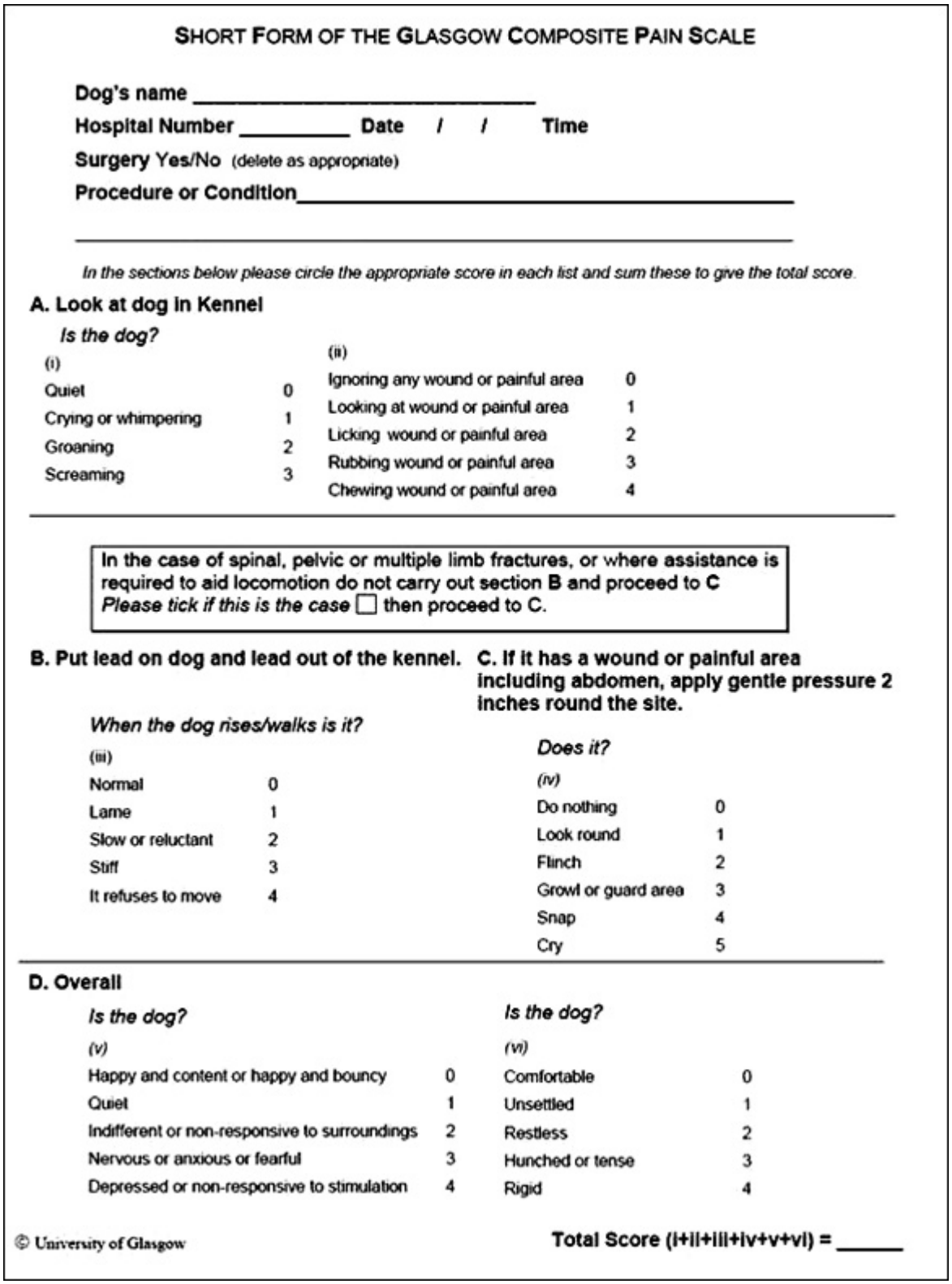
This study was carried out in a private veterinary clinic specialized in neurosurgery. All the dogs diagnosed with an acute thoracolumbar intervertebral disc extrusion undergoing hemilaminectomy were enrolled. Animals suffering from pre-existing painful diseases (like osteoarthritis or medical painful conditions) were excluded. This study and procedures were approved by the Ethics Committee of the Department of Veterinary Sciences, University of Messina, Italy. The research was carried out in a high standard veterinary referral clinic, and client-owned dogs were enrolled after the informed consent had been provided by the owners.

Fifty dogs of different breeds (21 Dachshunds, 6 mongrel dogs, 3 Beagles, 2 Miniature poodles, 2 Chihuahuas, 2 Jack Russell terriers, 2Beijingers, 1 Yorkshire terrier, 1 Rottweiler, 1 Staffordshire, 1Dalmatian, 1 English bulldog, 1 Maltese, 1 Zwergpinscher, 1 German shepherd, 1 Schnauzer, and 1 Shih tzu), gender (34 males and 16 females), age (ranging from 18 months to 14 years; mean ± standard deviation [SD]: 5.7±2.8 years), and body weight (ranging from 2.5 to 45 kg; mean ± SD: 10.4± 8.6 kg), met the criteria required. Each dog was subjected to clinical and neurologic examinations, haematological and haematochemical profiles, radiographs of the spine, CT (Toshiba Asteion VR; Toshiba America Medical Systems, Tustin, California, USA), and mielo-CT to obtain a definitive diagnosis of acute thoracolumbar intervertebral disc extrusion at the thoracolumbar region (T13-L3). All the animals underwent hemilaminectomy as early as possible. Perioperative narcosis and analgesia were obtained in all patients with the same anaesthetic protocol: acepromazine (Prequillan; Fatro SpA, Ozzano Emilia, BO, Italy) 0.02 mg/kg intramuscularly (IM) for the premedication, propofol (Rapinovet®, Intervet Italia Srl, Milano, MI, Italy) 3 mg/kg intravenously (IV), and fentanil (Fentanest; Pfizer Italia Srl, Latina, Italy) 0.004 mg/kg IV for the induction, and isoflurane (Isoflurane Vet; Merial Italia SpA, Noventa Padovana, PD, Italy) and oxygen for the maintenance. During the whole surgery, analgesia was provided by fentanil (0.004 mg/kg/h IV) in constant rate infusion. A broad-spectrum antimicrobial therapy was started. All the surgeries lasted approximately 60 minutes.

At the end of the surgery, dogs were randomly divided into 2 groups to receive different postoperative analgesic treatment. Group A dogs (n = 25) were treated with tramadol (Altadol; Formevet SpA, Milan, Italy) 3 mg/kg IM every 6 hours,whereas group B dogs (n = 25) were treated with buprenorphine (Bupaq Multidose; Richter Pharma AG, Wels, Austria) 0.02 mg/kg IM every 8 hours. Ten minutes before the end of the surgery, constant rate infusion of fentanil was stopped, and the first IM administration of the chosen analgesic was performed by an operator who was unaware of the contents of the syringes. All patients were

monitored until fully awake and then hospitalized for 48 hours. Dogs were housed in an air-conditioned and quiet intensive care unit with soft lights and natural photoperiod. External stimuli that could have worsened the dogs' stress because of their painful condition were minimized. Dogs were hospitalized in specific cages of adequate dimensions for each dog size to restrict movement on a soft and comfortable surface. Fluid balance, antibiotic administration, gastrointestinal motility, nutrition, nursing care, and wound care were managed.

The short form of the Glasgow Composite Pain Scale (GCPS-SF) was used for the evaluation of the degree of pain in all patients (Reid et al., 2007). The short form of the Glasgow Composite Measure Pain Scale (GCMPS-SF) comprises 6 behavioural categories with associated descriptive expressions (items): section A−(1) vocalization (score, 0-3) and (2) attention to wound (score, 0-4); section B−(3) mobility (score, 0-4); section C−(4) response to touch (score, 0-5); and section D−(5) demeanour (score, 0-4) and (6) posture/activity (score, 0-4). Items are placed in increasing order of pain intensity and numbered accordingly. The operator chooses that item within each category that best describes the dog's behaviour, and ranked scores are summed (total score: 1 + 2 + 3 + 4 + 5 + 6); the maximum pain score is 24 or 20 if mobility is impossible to assess (Figure 1).



SHORT FORM OF THE GLASGOW COMPOSITE PAIN SCALE

Dog's name _____

Hospital Number _____ **Date** / / **Time**

Surgery Yes/No (delete as appropriate)

Procedure or Condition_____

_____

In the sections below please circle the appropriate score in each list and sum these to give the total score.

A. Look at dog in Kennel

Is the dog?

(i)

Quiet	0
Crying or whimpering	1
Groaning	2
Screaming	3

(ii)

Ignoring any wound or painful area	0
Looking at wound or painful area	1
Licking wound or painful area	2
Rubbing wound or painful area	3
Chewing wound or painful area	4

In the case of spinal, pelvic or multiple limb fractures, or where assistance is required to aid locomotion do not carry out section B and proceed to C
Please tick if this is the case ☐ **then proceed to C.**

B. Put lead on dog and lead out of the kennel.

When the dog rises/walks is it?

(iii)

Normal	0
Lame	1
Slow or reluctant	2
Stiff	3
It refuses to move	4

C. If it has a wound or painful area including abdomen, apply gentle pressure 2 inches round the site.

Does it?

(iv)

Do nothing	0
Look round	1
Flinch	2
Growl or guard area	3
Snap	4
Cry	5

D. Overall

Is the dog?

(v)

Happy and content or happy and bouncy	0
Quiet	1
Indifferent or non-responsive to surroundings	2
Nervous or anxious or fearful	3
Depressed or non-responsive to stimulation	4

Is the dog?

(vi)

Comfortable	0
Unsettled	1
Restless	2
Hunched or tense	3
Rigid	4

Total Score (i+ii+iii+iv+v+vi) = _____

Figure 1. Short Form of Glasgow Composite Pain Scale (GCPS-SF) in dogs

The compilation of questionnaires was performed by the same operator, who was unaware of group allocation, to avoid a possible inter-observer variability and to minimize the effect of subjective bias. Pain was evaluated during the preoperative and, then, at 2, 12, and 24 hours after surgery. Forty-eight hours after surgery, the patients were discharged.

The drugs administered in this trial are registered for use in dogs.

Pain scores obtained using GCPS-SF were reported as mean ± SD, median and range. Data were tested for normality using the Shapiro-Wilk normality test. Data did not result to be normally distributed ($P > 0.05$) and were analyzed using the Mann-Whitney U test for nonparametric analysis. Significance was set at $P < 0.05$. No difference in scores between the 2 groups was taken as the null hypothesis.

Analgesic activity of tramadol and gabapentin in dogs with Degenerative Lumbosacral Stenosis (DLSS, cauda equina syndrome)

This study was carried out in a high standard veterinary referral clinic specialized in neurosurgery. All dogs diagnosed with degenerative lumbosacral stenosis (DLSS) that, based on clinical conditions or on the owner's decision, underwent a conservative medical treatment, were enrolled in the study, after the informed consent had been provided by the owners. Animals that were diagnosed, based on the history and clinical examination, to be affected by concomitant painful diseases were excluded from the study.

Ten dogs of different breeds (2 Labrador Retriever; 2 mongrel dogs; 1 Boxer; 1 Collie; 1 Kurzhaar; 1 Samoiedo; 1 Siberian Husky; 1 Newfoundland), gender (5 males and 5 females), age (ranging from 2,5 to 11,5 years; mean±SD: 6,9±3,5 years) and body weight (ranging from 22 to 52 kg; mean±SD: 32,4±8,7 kg) met the inclusion criteria and were enrolled in the study. Among them, 70% (7/10) were companion animals while 30% (3/10) were agility dogs. All the dogs enrolled in the study were slightly to moderately overweight (Body Condition Score, BCS, ranging from 4.5/9 to 7/9: mean±SD: 6.1±0.9).

On first clinical evaluation, anamnesis regarding type and onset of clinical signs was recorded. Each dog underwent a neurological examination, in order to determine the localization of the lesion by assessing postural reactions and spinal reflexes of the hind legs. The hemistand, hemiwalk, hopping, conscious proprioception and wheelbarrow thoracic limbs tests, were performed in order to assess the conscious perception of body position in the space and to emphasize the possible weakness in the forelegs. The normality of these tests allowed to exclude, from the possible differential diagnosis, spinal injuries located at an upper level than the lumbo-sacral area. The evaluation of the spinal reflexes of the hind limbs, the patellar, cranial tibial muscle, sciatic nerve and withdrawal reflexes, was performed in order to evaluate the L4-S2 spinal tract. Abnormalities of the hind legs spinal reflexes together with normal postural reactions, provided a clinical diagnosis of a lumbo-sacral lesion and a suspect of DLSS.

All the dogs that had a clinical suspect of DLSS, based on history and neurological signs, were selected for Computed Tomography (CT) examination. Before the execution of the CT, all the dogs underwent a general physical examination, haematological and haematochemical profiles. After a fasting period of 12 hours, the dogs were anesthetized with the same anaesthetic protocol. Butorphanol (Dolorex®, Intervet Italia Srl, Milano, MI, Italy) 0,1 mg/kg IM and medetomidine (Domitor®, Pfizer Italia Srl, Milano, MI, Italy) 2 µg/kg IM were used for the premedication. The anaesthesia was induced with propofol 3 mg/kg IV 20 minutes after the premedication, while isoflurane was administered for the maintenance together with oxygen. When the dogs were in general anaesthesia, two radiographs of the spine in latero-lateral (LL) and ventro-dorsal (VD) projections were performed. Immediately after, a CT spiral scan (Toshiba, “Asteion VR”) examination of the L4-S1 spinal tract was started, with the patient in dorsal recumbency. When it was not possible to find an obvious hypo- or hyperdense lesion of the spinal tissue on the CT, a CT myelography (mielo-CT) of the same spinal segment was performed, in order to achieve a definitive diagnosis. An organic

triiodinated non-ionic contrast medium (Iohexol, Omnipaque® 300 mg I/mL, GE Healthcare) 0.1 ml/kg was injected in the subarachnoid space between L5 and L6, in order to highlight any intra or extrudural compression with the same density of the tissue itself. All the imaging studies were reviewed by the same specialized operator.

After the diagnosis of DLSS was achieved, a conservative medical treatment was started in all the dogs enrolled in the study. The surgical treatment was discarded because of specific clinical conditions of the dogs or of financial constraints of the owner. All patients were treated with prednisolone (Vetsolone® 5 mg, Bayer Spa, Div. Sanità Animale, Milano, MI, Italy) 0.5 mg/kg/die orally (OS) for 3 weeks and then on alternate days during the fourth week. A weight loss diet was suggested in order to reduce the postural stresses related to overweight. At the same time, all the dogs were treated with tramadol (Altadol®, Farmavet, Urbisaglia, MC, Italy) 3 mg/kg every 8 hours PO for 4 weeks. However, after the first week of treatment with tramadol, 5 dogs showed only a slight improvement of clinical and neurological conditions, without any significant improvement of lumbo sacral pain. Because of ethical reason related to the degree of pain experienced by the dogs and because it was not possible to prescribe at home any other opioid drug due to the restriction of prescription, tramadol administration was discontinued and it was decided to start a treatment with gabapentin (Gabapentin®, Teva Italia, Milano, MI, Italy) 10 mg/kg every 8 hours PO for other 3 weeks.

Each dog underwent neurological examinations during the follow-up after 2 and 4 weeks of treatment. Evaluations of muscle tone, conscious proprioception, withdrawal and patellar reflexes were recorded on the first examination before the conservative treatment was started (T0) and after 2 (T2) and 4 (T4) weeks of follow-up. For each parameter considered during the neurological examination at each time point, a verbal rating was assigned. For statistical analysis, each verbal rating was converted in a numerical score.

- Muscle tone: active (0), passive (1), muscle atrophy (2);

- Propioception: normal (0), decreased (1), absent (2), exaggerated (3);
- Withdrawal reflex: normal (0), decreased (1), absent (2), exaggerated (3);
- Patellar reflex: normal (0), decreased (1), absent (2), exaggerated (3);

The Short Form of the Glasgow Composite Pain Scale (GCPS-SF) was used for the evaluation of the degree of lumbosacral pain in all patients (Reid et al., 2007) and the score obtained at T0, T2 and T4 were recorded. Pain evaluation was performed by the same operator, who was unaware of group allocation, to avoid a possible inter-observer variability.

For the evaluation of the treatment efficacy, feelings of the owners about the behavioural changes and degree of pain of their animals were recorded.

The scores obtained in the group of dogs that came out to have a poorly managed pain with tramadol and that were consequently treated with gabapentin (Group A), were compared with the ones obtained in the group treated only with tramadol (Group B) for each time point. Moreover, the scores obtained in Group A after the first week of treatment with tramadol (T1) were compared with the ones obtained after the following week of treatment with gabapentin (T2).

Descriptive statistic (range, mean and standard deviation, SD) was applied for age and body weight. Data were analyzed for normal distribution with Shapiro-Wilk normality test and came out to be not normally distributed ($p > 0.05$). Mann-Withney U Test and Wilcoxon signed rank test were used to evaluated the effect of the treatment and time on the evaluated parameters (muscle tone, conscious proprioception, withdrawal and patellar reflexes, and GCMPS-SF scores). Statistically significance was set at $p<0.05$.

All data collected during the trials were entered into a spreadsheet (Microsoft Excel); the statistical tests were performed using the STATISTICA 7 software (Stat Soft, Inc, Tulsa, OK; 2003).

4.2.3 Results

Comparison of the analgesic activity of buprenorphine and tramadol in dogs undergoing hemilaminectomy

Section B (mobility) of GCPS-SF was not carried out as a result of the animals' physical condition, so that the total score was out of 20 rather than 24.

No statistically significant difference in GCPS-SF scores between the 2 groups was recorded before surgery ($P > 0.8$), showing homogeneity of the sample, characterized by a high degree of pain (Group A: 15.92±1.58, median: 16, range: 12-18; Group B: 15.84±1.62, median: 16, range: 13-18). Table 1 shows the descriptive statistic of GCPS-SF scores calculated for the two groups.

Statistical analysis showed a significant effect of time both within and between the groups. Both in Group A (tramadol) and Group B (buprenorphine), GCPS-SF score was significantly higher preoperatively compared with all other time points of the postoperative period ($P < 0.001$), with lowest score values at 12 hours postoperatively and 24 hours postoperatively compared with 2 hours postoperatively (Figure 2). The statistical analysis also showed a significant effect of medication, with GCPS-SF scores lower in dogs treated with buprenorphine than in those treated with tramadol ($P = 0.049$, $P = 0.016$, and $P = 0.003$ at 2 hours postoperatively, 12 hours postoperatively, and24 hours postoperatively, respectively) (Figure 3).

In detail, during the period of monitoring, group B dogs showed greater comfort, with less tendency to impatience, less spinal stiffness, and higher tolerance of handling than those of Group A, as soon as 2 hours postoperatively after surgery. Similarly, after 12 and 24 hours postoperatively, both groups responded positively to the treatment, but Group B animals were found to be more manageable; they had a less capricious appetite (were less choosy with food), less tendency to nervousness, and kyphosis was rarely present. During the postoperative period, the GCPS-SF scores did not exceed 5 out of 20 in Group B (mean ± SD:

3.12±1.08; median: 3; range: 1-5), whereas 2 dogs at 2 hours postoperatively and 1 dog at 12 hours postoperatively of group A scored 6 out of 20 (mean ± SD: 3.96±1.10; median: 4; range: 2-6), considered as a possible analgesic intervention level (Reid et al., 2007).

Table 1. Descriptive statistic of Glasgow Composite Pain Scale – Short Form (GCPS-SF) scores calculated for Group A and Group B (mean, standard deviation [SD], median, minimum [Min], maximum [Max], lower and upper quartiles, and variance) during the preoperative period (pre-op) and 2 (2 hours post-op), 12 (12 hours post-op), and 24 (24 hours post-op) hours after surgery

Group	Time	Mean	SD	Median	Min	Max	Lower Quartile	Upper Quartile	Variance
A	*pre-op*	15.92	1.58	16	12	18	15	17	2.49
	2hpost-op	4.56	0.77	5	3	6	4	5	0.59
	12hpost-op	4.32	0.83	4	3	6	4	5	0.68
	24hpost-op	3	0.99	3	2	5	2	3	0.98
B	*pre-op*	15.84	1.62	16	13	18	15	17	2.64
	2hpost-op	3.88	0.59	4	3	5	3.5	4.2	0.34
	12hpost-op	3.48	0.59	3	2.5	4.5	3	4	0.34
	24hpost-op	2	0.92	2	1	4	1	2.5	0.85

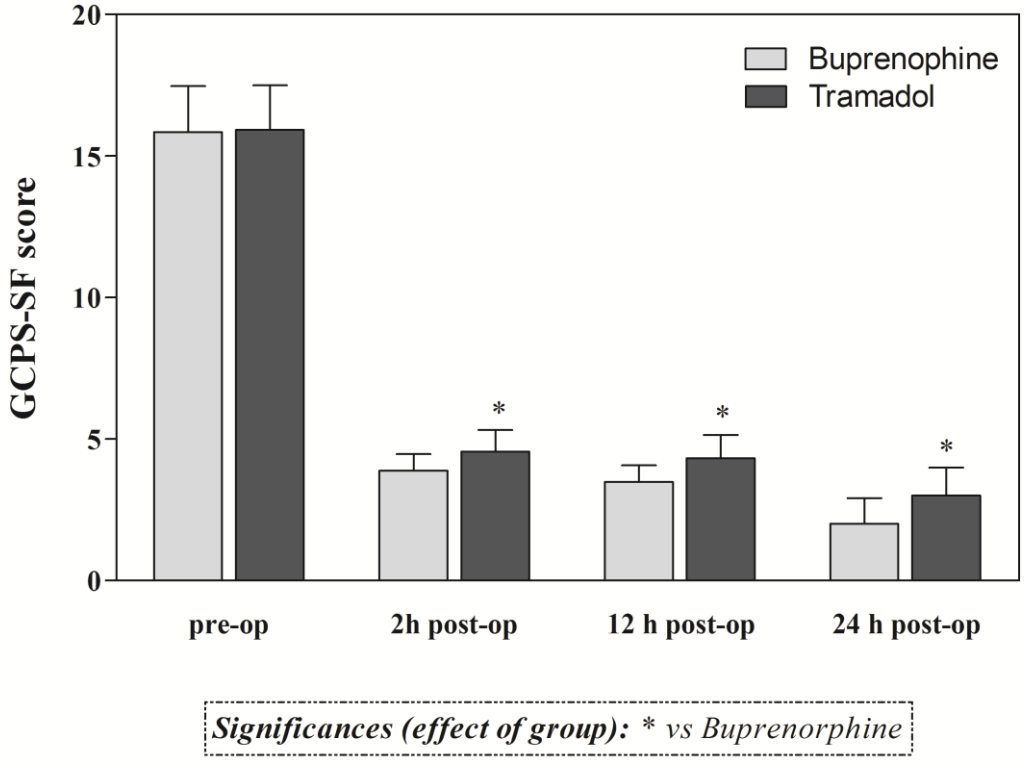


Figure 2. The Short Form of the Glasgow Composite Pain Scale (GCPS-SF) score evaluated in Group A (tramadol) and Group B (buprenorphine) before surgery (preoperatively) and during postoperative period (2 hours postoperatively, 12 hours postoperatively, and 24 hours postoperatively) with the respective significance found between groups. Pre-op, preoperatively; post-op, postoperatively

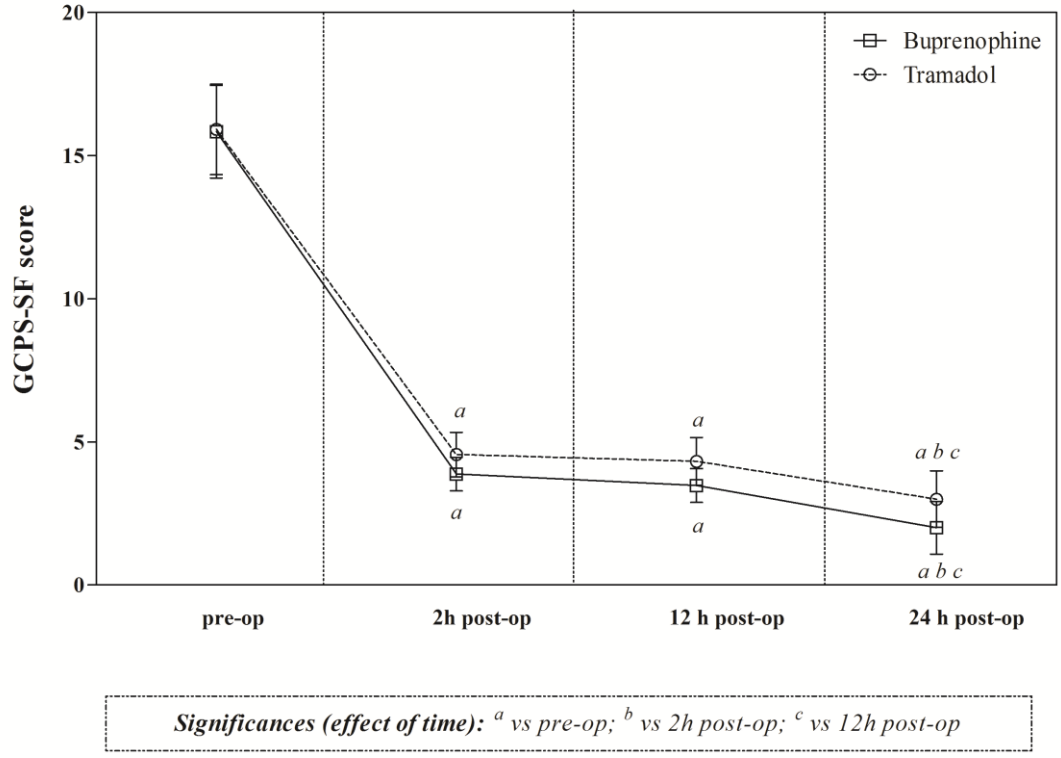


Figure 3. The Short Form of the Glasgow Composite Pain Scale (GCPS-SF) score evaluated in Group A (tramadol) and Group B (buprenorphine) before surgery (preoperatively) and during postoperative period (2 hours postoperatively, 12 hours postoperatively, and 24 hours postoperatively) with the respective significances found within each group. Pre-op, preoperatively; post-op, postoperatively

Analgesic activity of tramadol and gabapentin in dogs with Degenerative Lumbosacral Stenosis (DLSS, cauda equina syndrome)

The most common clinical signs reported by owners were weakness, back pain, mono- or bilateral hind legs lameness, reluctance to jump, climb the stairs and sit down. According to the owners, clinical signs had started from 3 days to 2 months before (mean±SD: 17.7±19 days). No one of the dogs enrolled in the study has showed urinary or faecal incontinence.

Postural tests did not show any propioceptive deficit in all the dogs enrolled in the study, allowing to exclude spinal injuries above the lumbosacral tract. Moreover, the conscious proprioception remained normal (score 0) in all the dogs at all time points, so statistical analysis was not applied.

On the first clinical evaluation, evoked pain on palpation of the L7-S1 tract of the spine and mild to severe mono or paraparesis of hind limbs was found in all the dogs (10/10). Muscle tone appeared reduced in 70% of dogs (7/10), while the evaluation of patellar reflex showed pseudo-hyperreflexia in 50% of the sample (5/10) and a marked slowdown in the withdrawal reaction of the hind limbs in 70% of dogs (7/10). All this changes were indicative of a localization of the lesion in the segment L4-S1.

Haematological and haematochemical profiles were within the laboratory ranges in all animals and no adverse reactions have occurred after the administration of the contrast medium.

On radiological examinations, abnormalities that could have suggested a DLSS have been found only in 30% of dogs (3/10). In particular, the LL projection showed subchondral sclerosis of the endplates L7-S1, increased radiopacity of the intervertebral space of L7-S1 and spondylolisthesis, respectively.

The CT examination allowed to achieve a definitive diagnosis of DLSS in 50% of the dogs, while in the remaining 50% it was necessary to perform a CT myelography (Figure 4). Slight disc bulging L7-S1, was found in 40% of dogs (cases 5, 6, 7 and 9), disc protrusion L7-S1

(Figure 5), with dorsal deviation of the dural sac, in the 30% (cases 3, 4 and 8), retrolisthesis of S1, in the 30% (cases 2, 4 and 7), mono or bilateral, mild to moderate foraminal stenosis (Figure 6), with the presence of hyperdense material in the foraminal space, in the 60% (cases 2, 3, 5, 6, 7 and 8), severe sclerosis of the endplates, with alterations of their profile, in the 50% (cases 1, 6, 7, 8 and 10) and discospondylitis L7-S1, in 10% of dogs (case 1) (Table2).

Table 2. Localizations and types of lesions detected on Computed Tomography (CT) examination in the 10 dogs enrolled in the study

Case	Neurological lesions
1	Severe lysis of the endplates and the vertebral body L7-S1. Discospondylitis
2	Stenosis of the left intervertebral foramen (L7-S1) and retrolisthesis.
3	L7-S1 disc protrusion, with dorsal deviation of the *cauda equina*. Slight decrease of the foraminal space.
4	Protrusion L7-S1. Decrease of the dural fat. Retrolisthesis S1. Foraminal spaces preserved.
5	Stenosis L7-S1. Severe stenosis of the intervertebral foramina due to large osteophytosis. *Cauda equina* deviated dorsally. Protruding disc L7-S1.
6	Protruding disc L7-S1, which deviated dorsally the dural sac and invaded the intervertebral foramen. Sclerotic endplates and hyperdense appearance of the spinal cord.
7	Protruding disc L7-S1 and bilateral foraminal stenosis with hyperdense material in the right foramen. Retrolisthesis S1 with alteration of the profile of the endplates and deforming spondylarthrosis.
8	Severe alteration of the endplates at L7-S1, which appeared hypodense.
9	Slight protrusion disc L7-S1, which deviated dorsally the dural sac, without altering the foraminal spaces.
10	Reduction of the intervertebral space L6-L7. Extradural compression with dorso-lateral deviation of the *cauda equina*. Subchondral sclerosis of the endplates.

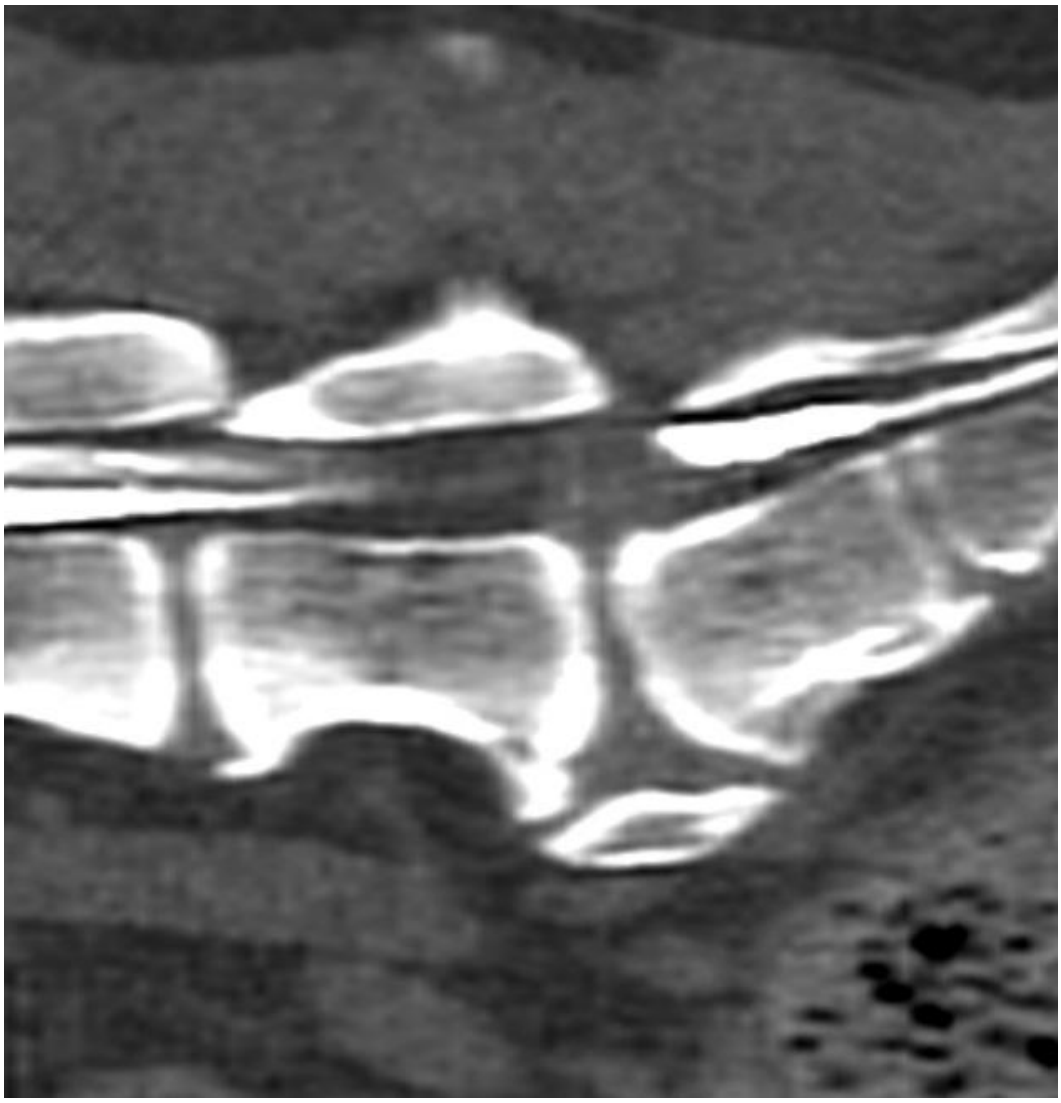


Figure 4. Disappearance of the contrast lines on CT myelography due to a severe compression of the dural sac L7-S1 (case 1)

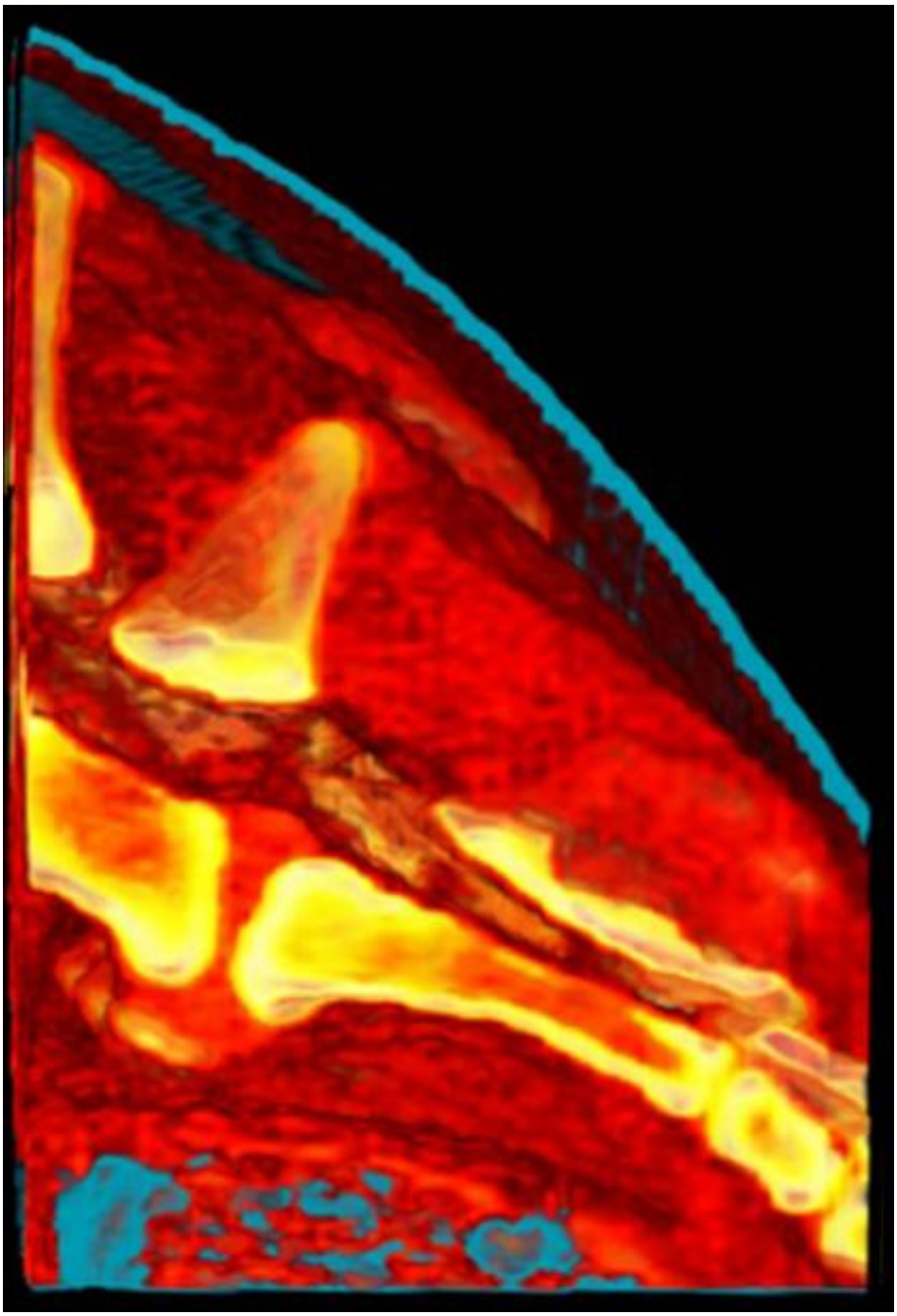


Figure 5. 3D reconstruction (soft tissues algorithm) of a Computed Tomography (CT) image of disc protrusion L7-S1 (case 3)

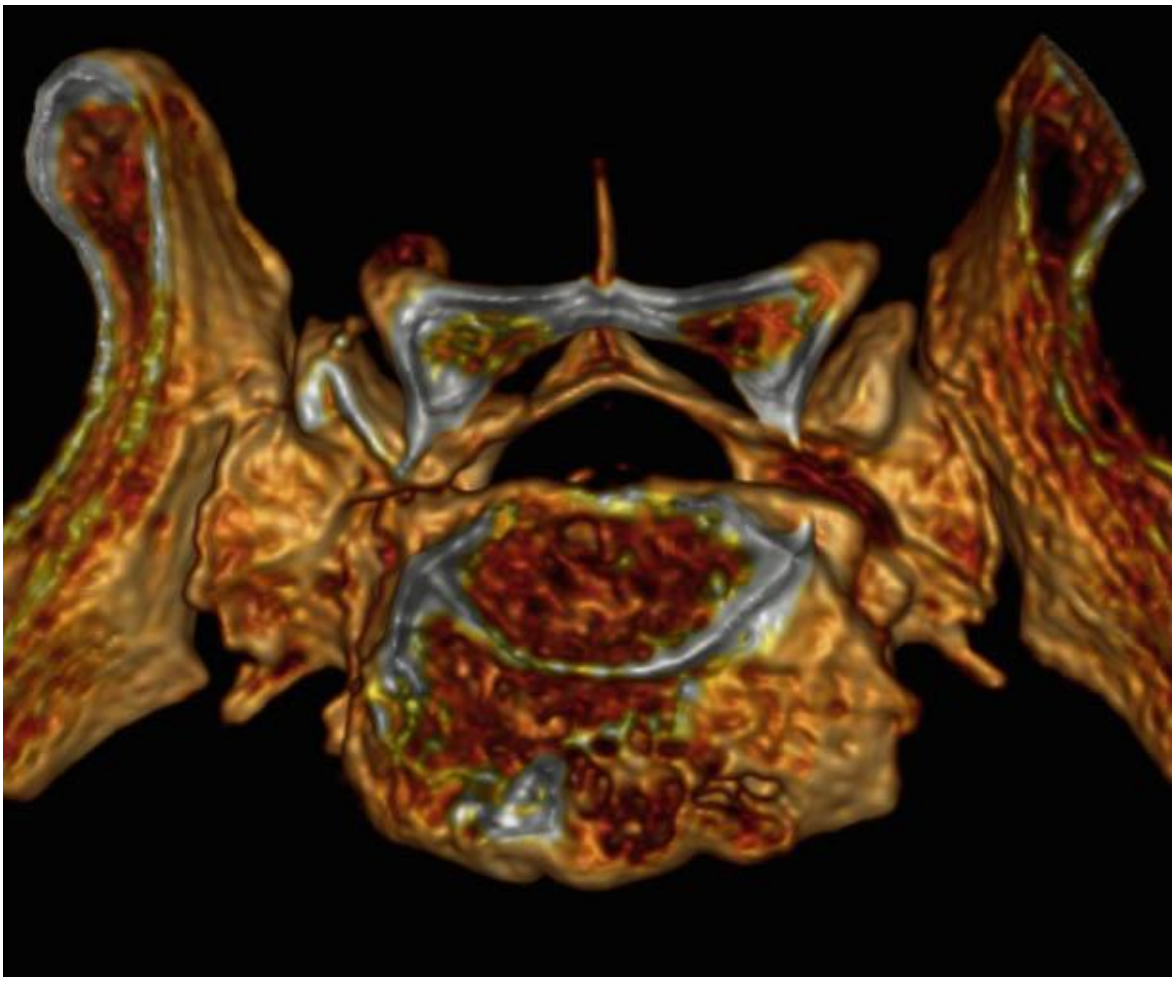


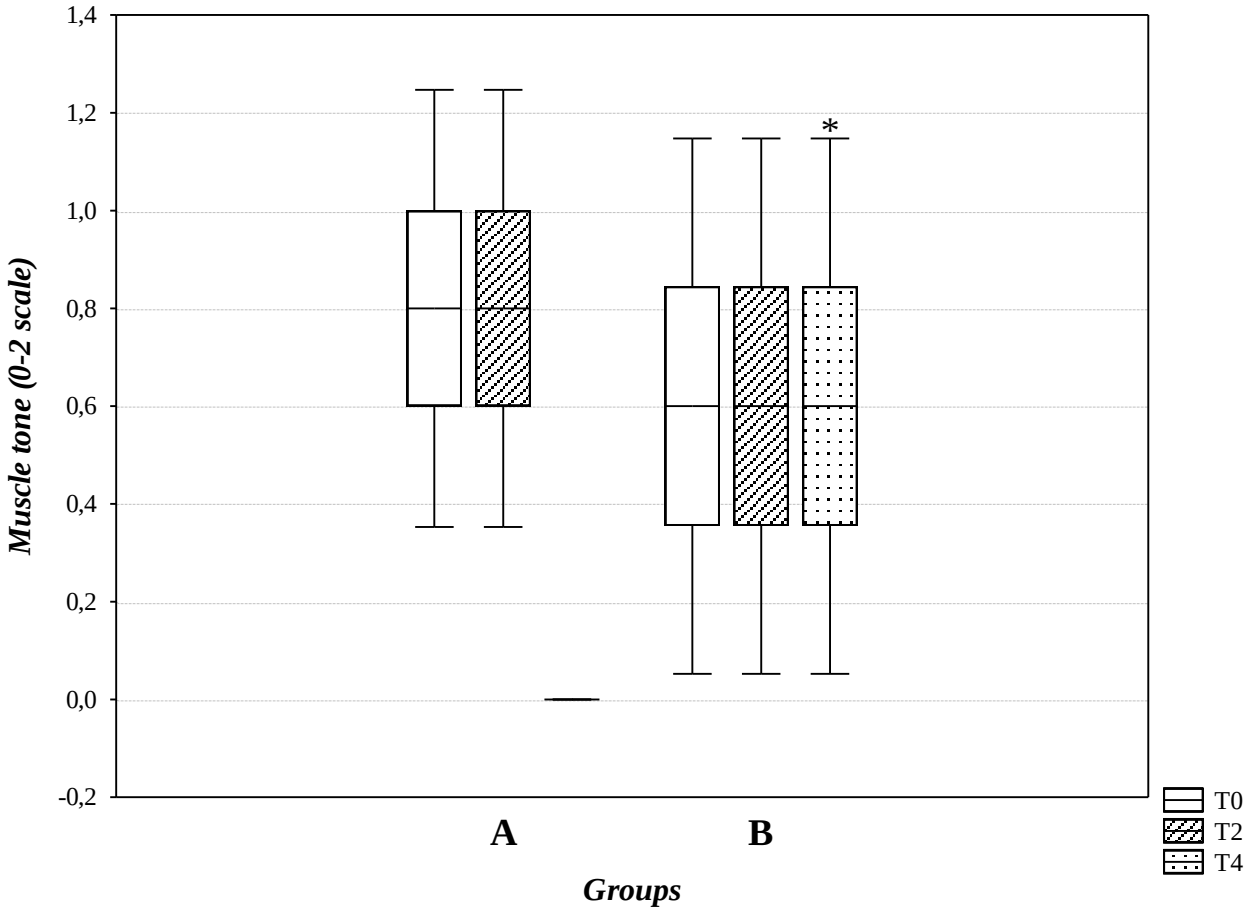
Figure 6. 3D reconstruction (bone tissue algorithm) of a Computed Tomography (CT) image in transverse section of moderate foraminal stenosis (case 2)

Statistical analysis showed a significant effect of time on muscle tone and lumbo-sacral pain, but not on withdrawal and patellar reflexes. In particular, statistical analysis showed a significant effect of the treatment on muscle tone in Group A at T4 ($p < 0.05$) but not at T2 in both groups and at T4 in Group B (Figure 7).

Regarding lumbo sacral pain assessment, section B (mobility) of GCPS-SF was not carried out because all the animal showed different degree of paraparesis of the hind legs, so that the total score was out of 20 rather than 24.

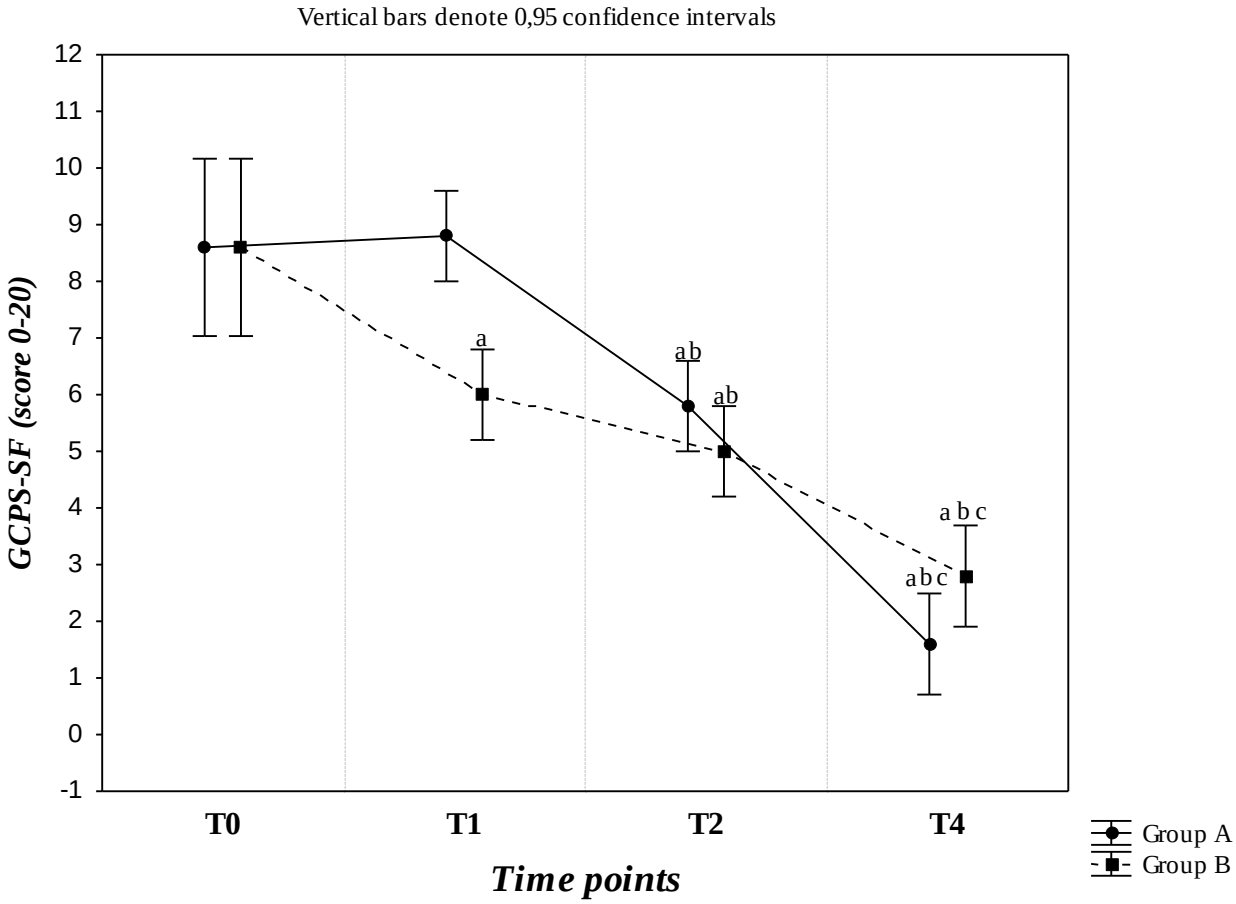
No statistically significant difference in GCPS-SF scores between the 2 groups was recorded at first clinical evaluation (T0) ($P > 0.9$), showing homogeneity of the sample, characterized by a moderate degree of pain (Group A: mean ± SD: 8.6±1.1; median: 9; range: 7-10; Group B: mean ± SD: 8.6±1.8; median: 8; range: 7-11).

A statistically lower GCPS-SF score at T1 was found in Group B compared to Group A ($p = 0.009$). For this reason, the dogs of Group A were considered to be non-responder to tramadol. However, after the analgesic treatment of Group A has been switched to gabapentin, no statistical differences have been found between the two groups both at T2 and at T4 ($p > 0.05$). Moreover, a statistical effect of time has been found within both groups in all the time points (Group A: $p = 0.04$ at T0 vs T2 andT4, T1 vs T2 and T4, and T2 vs T4; Group B: $p = 0.04$ at T0 vs T1, T2 and T4, T1 vs T2 and T4, and T2 vs T4), with the exception of T1 in Group A ($p = 0.59$) (Figure 8). Already after the 2nd week of treatment, GCPS scores were considerably lower in both groups (Group A: mean±SD: 5.8±0.8, median:6, range: 5-7; Group B: mean±SD: 5±0.7, median: 5, range: 4-6), but still around the possible intervention level (6 out of 20). After 4 weeks of treatment, all the dogs showed a further reduction in GCPS score (Group A: mean±SD: 1.6±0.9, median:1, range: 1-3; Group B: mean±SD: 2.8±0.8, median: 3, range: 2-4).



*Statistical significance: * vs Group A (p <0.05)*

Figure 7. Muscle tone score (0-2 scale) evaluated in Group A (gabapentin) and Group B (tramadol) after the first clinical evaluation (T0) and after 2 (T2) and 4 (T4) weeks of treatment with the respective significance found between the two groups



Statistical significance: a vs T0 ($p < 0.05$), b vs T1 and c vs T2

Figure 8. Glasgow Composite Pain Scale – Short From (GCPS-SF) scores evaluated in Group A (gabapentin) and Group B (tramadol) after the first clinical evaluation (T0) and after 2 (T2) and 4 (T4) weeks of treatment, with the respective significance found within the groups

4.2.4 Discussion

The control of pain is part of a veterinarian's duty of care toward their patients to ensure animal welfare. Painful sensations determine alterations of various body systems, including cardiovascular, respiratory, gastro-enteric, and immune systems, and slow down the tissue healing process. Pain is also known to impair decision making and mental processing and can determine behavioural changes such as vocalization, anxiety or depression, aggressiveness, and automutilation of the affected area. Behavioural changes that result in aggression and anxiety are potentially dangerous for the veterinarians, making it more difficult to manage and examine the dog, and may lead to a drastic reduction of animal welfare and an impairment of patient's capability to cope in stressful conditions. Although the importance related to the alleviation of pain in companion animals is increasing, recent surveys suggested that the use of analgesic drugs in small animal veterinary practice is suboptimal (Reid et al., 2007). In fact, pain management is a real challenge in veterinary medicine because it requires special skills of the clinician in recognizing, as objectively as possible, its different aspects in nonverbal patients. The GCMPS is a validated behaviour-based composite scale, developed using psychometric methodology, to assess acute pain in dogs. This scale measures pain to a level of precision suitable for clinical trials (Morton et al., 2005). Indeed, the application of a scaling model is particularly important in quantitative studies of analgesia, for example, in pre-, peri-, and postoperative settings. A short form of the GCMPS-SF was developed for routine clinical use, where the emphasis is on speed, ease of use, and guidance for analgesia provision (Reid et al., 2007). Although many researchers (Lascelles and Waterman, 1997) support the need to include, in analgesic drug studies, a control group without treatment, it was decided not to use a comparison group for ethical reasons related to the professional code of ethics that ensures, first of all, the physical and mental welfare of the animal. In fact, it is widely recognized that disc and spine pathological conditions and surgeries are characterized by moderate to severe degree of pain (Carroll and Martin, 2007), as confirmed by the high

scores achieved during the first evaluations of our samples. Likewise, other authors compared the effect of morphine and buprenorphine (Brodbelt et al., 1997) and tramadol and morphine (Mastrocinque and Fantoni, 2003) administered for postoperative pain in dogs, without entering a control group.

In the first study, both buprenorphine and tramadol showed good efficacy in controlling acute postoperative pain resulting from hemilaminectomy, as evidenced by the rapid and significant improvement of the GCPS-SF scores. However, buprenorphine showed a higher and more rapid analgesic effect than tramadol. Although tramadol has a lower affinity for the μ-receptors, several studies in humans have shown an analgesic effect of tramadol similar to that of morphine (Matrocinque and Fantoni, 2003; Neves et al., 2012; Kongara et al., 2013). However, some studies carried out in the canine species suggest a lower efficacy than in man. Particularly, in beagle dogs, a limited production of the metabolite M1, which has a high affinity for μ-opioid receptors, and of spinal serotonin receptors, has been proven (Wu et al., 2001). Even in rats, tramadol showed an analgesic efficacy lower than buprenorphine (McKeon et al., 2011).

At the dosage and condition of administration used in this study, both molecules showed no side effects; in particular, no depressant effects were observed on the respiratory function, as recorded in other studies performed in dogs with tramadol and morphine (Mastrocinque and Fantoni, 2003).

The advantages of treatment with buprenorphine include a greater plasma half-life (from 6 to 8 hours), with a lower frequency of administration, good analgesic effect, and different routes of administration. Disadvantages include the ceiling effect, the difficulties of antagonization, and the administration subjected to the restriction for the narcotic drugs.

The advantages of the use of tramadol include a lower occurrence of adverse respiratory, gastrointestinal, and immunomodulatory effects compared with opioid drugs, and an anti-inflammatory effect, albeit moderate (Buccellati et al., 2000).

The results obtained in the present study show that both buprenorphine and tramadol can be safely used to control postoperative pain in dogs treated for acute intervertebral disc extrusion, although buprenorphine has shown a faster and higher analgesic effect. Buprenorphine might be better than tramadol during the first stage of hospitalization, but tramadol might represent a good alternative to continue treatment at home, considering its lower incidence of side effects. In conclusion, the findings of this study seem to suggest that the use of both medications can contribute to improve animal welfare and provide pain relief.

The results of second study are in accordance with those described in the literature regarding the epidemiology of lumbosacral degenerative stenosis in dog. All the dogs affected by DLSS were of middle sized to large breed, with a mean age of 6.9 years, as it was already described in previous studies (De Risio et al., 2001; Suwankong et al., 2008), and most of them also were in an overweight condition. Unlike other studies, there were no German Shepherds among the dogs enrolled in this study, despite this is a common breed in the South of Italy where the study was carried out. However, this can be due to the small number of animals enrolled. Moreover, the number of male and female dogs was exactly the same, but this can also be due to the limited dimension of the population under study. Clinical signs and time of onset before the first clinical evaluation were comparable to previous studies, with lameness, back pain and difficulty to jump and sit down the most commonly reported (Suwankong et al., 2008).

Advanced diagnostics, such as computed tomography or CT myelography, had been essential for the diagnosis of cauda equina syndrome. Only in three cases radiological examination provided a suspect of DLSS, while in half of the patients it was necessary to perform a CT myelography to confirm the diagnosis. As already described in the veterinary literature on DLSS (Suwankong et al., 2006), the most common abnormalities detected on CT scan were disc bulging L7-S1, disc protrusion L7-S1, mono or bilateral, mild to moderate foraminal

stenosis, with the presence of hyperdense material in the foraminal space, severe sclerosis of the endplates, with alterations of their profile, and discospondylitis.

In both groups no side effects associated with the two treatments have occurred. However, 5 of the dogs enrolled in the study showed no statistical significant reduction of GCPS score for lumbosacral pain after one week of treatment with tramadol, compared to the others 5. Although one week could have been a period not long enough to determine an improvement in pain score, the decision to start gabapentin for pain management in these dogs was due to ethical reasons related to the moderate degree of pain experienced, which was determining a poor quality of life, as reported also by the owners. It was not possible, due to the restriction of prescription for opioid drugs, to start a treatment with an opioid, considering that the dogs were not hospitalized. NSAIDs were not chosen because the dogs were already under treatment with a corticosteroid, so it was probably not safe to combine two classes of drugs with the same side effects, especially on the gastrointestinal tract. The use of gabapentin is widely recognized in human as a treatment for neuropathic pain (Alvarado and Navarro, 2016; Akkurt et al., 2015;) in general, and with lumbo sacral stenosis in particular (Yaksi et al., 2007). Moreover, although not being registered for the use in companion animals, several reports exist on the use of gabapentin in animal models of neuropathic pain (Mangaiarkkarasi et al., 2015), in dogs undergoing amputation of a forelimb (Wagner et al., 2010), mastectomy (Crociolli et al., 2015), and for postoperative pain after intervertebral disc surgery (Aghighi et al., 2012). Although the pharmacokinetic studies carried out in dogs and cats have shown a terminal half life of about 3 hours (KuKanich and Cohen, 2011; Siao et al., 2010), based on other studies on the use of gabapentin in animals, it was decided to administer the gabapentin every 8 hours. In fact, as reported by Kukanich and Cohen (2011), plasma concentration of gabapentin should remain above that level that is, at least in human, the effective one, within the first 8 hours after administration. The dose used was the same reported in previous studies (10 mg/kg). However, in both studies where gabapentin was administered to treat

postoperative pain after intervertebral disc surgery (Aghighi et al., 2012) and mastectomy (Crociolli et al., 2015), without any significant improvement of pain scores, it was administered every 12 hours, that could have been a too low frequency of administration to guarantee pain relief.

The significant reduction of pain score that has been observed in the group treated with gabapentin, could also be due to the fact that the dogs enrolled in this study did not have any proprioceptive deficit or urinary and/or faecal incontinence that have been reported to be related with a worse outcome in other studies (Suwankong et al., 2008). However, also the group treated only with tramadol did not show any proprioceptive deficit and had the same pain score on admission, showing homogeneity of the two groups on first examination.

Limitations of this study were the small number of dogs enrolled and the lack of a longer follow-up period. Moreover, considering that the dogs did not undergo surgery, it was not possible to confirm the correspondence with diagnostic findings. However, in previous study it has been shown that CT scan is a reliable method for DLSS diagnosis, with a good correspondence with surgical findings (Suwankong et al., 2006).

The results of this study seem to suggest that both tramadol and gabapentin could be two effective treatments in reducing lumbosacral pain in dogs with DLSS. However, some dogs can be non responders to tramadol. This could be due to a different response of each specific individual to the drug or to the fact that, in some patients, tramadol could be not properly metabolized and need a longer period to reach its full activity. However, just after one week from the beginning of the treatment, the group treated with gabapentin showed the same pain score compared to the one treated with tramadol. This seems to suggest a faster onset of gabapentin analgesic activity compared to tramadol. The rapid reduction in pain score is also supported by the considerations of the owners on the resumption of normal behaviours and physical activity. However, this fast improvement could also be due to the fact that Group A had already received tramadol for one week, even if without any obvious improvement.

Moreover, the improvement of muscle tone in the group treated with gabapentin after 4 weeks of follow-up could be due to the resumption of a moderate physical activity determined by a better pain control than in dogs treated with tramadol.

Synergic effects were probably associated with the anti-inflammatory effect of prednisolone, that promotes vascular supply and reduces the compression on nerve roots, with motion restraint, to minimize the risk of sudden movements that could cause an excessive stress of the spine, and with the reduction in body weight. The persistence of abnormal spinal reflexes after 4 weeks of treatment was an expected result, since the medical treatment did not solve the underlying problems and the drugs used had an analgesic effect but not a neurotrophic one.

Although gabapentin has been used from several years for neuropathic pain control in human medicine, references of controlled clinical trials are still lacking in the veterinary literature. Further studies should be carried out on a wider number of animals in order to confirm the analgesic activity of gabapentin in veterinary patients suffering from DLSS and other painful neurological conditions of the peripheral and central nervous system and to rule out eventual adverse affects related to prolonged administration.

CONCLUSIONS

Human as well as veterinary patients may be admitted to an Intensive Care Unit (ICU) because of different pathological conditions, such as hypovolemia, circulatory failure, respiratory distress, intoxications, trauma and severe pain. However, all these and other conditions that may require an intensive monitoring and treatment have in common the severity of the disease which represents a life-threatening hazard for the patient, as described in the cases reported in this thesis. Many organs and systems can be involved, even in the same critical patient, through different pathological mechanisms. However, all the possible critical conditions could end up in the patient's death if not promptly and properly managed.

Considering the variety of pathological conditions that could be found, the ICU doctor should have knowledge on different medical fields, from internal medicine to surgery, and should be able to perform a quick but careful physical examination of the patient, to choose the right diagnostic tests in order to confirm or exclude the possible differential diagnosis, and to start the proper treatment. Moreover, a close monitoring is primary for the management of a critical patient, in order to detect any possible complication, as soon as it eventually occurs. The activity of a veterinary ICU doctor is even more complicated due to the fact that veterinary patients are not able to refer what is the cause of their illness and how it started, and that sometimes, the previous history of the animal is unknown (for example, for stray dogs and cats). For this reason, the veterinary ICU doctors has to constantly improve their knowledge through a deep study and specialist training courses. However, working experience is also one of the most important tools for ICU doctors in order to make them able to identify and treat every conditions or complications that may affect an ICU patient.

"Time is money", people say, but in a critical patient it can make the difference between life and death. Therefore, time is also life, and this is probably the aphorism that better fits to the field of Emergency and Critical Care medicine.

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