UNIVERSITÀ DEGLI STUDI DI NAPOLI "FEDERICO II"

PhD Thesis

"New applications for the *One Health: Healthcare Zooanthropology* and the Federico II Model of Zootherapy"

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Fame is a bee.
It has a song -
It has a sting -
Ah, too, it has a wing.

Emily Dickinson

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Valerio, the only possible love.

List of abbreviations

AAIs	animal assisted interventions
AAT	animal assisted therapy
AAA	animal assisted activity
AAE	animal assisted education
AMA	american medical association
AVMA	american veterinary medical association
CSEN	national educational sports centre (cinophilia)
DEC	dog educational centres
IRE	interspecific relationships expert
GCs	glucocorticoids
HAI	human animal interaction
HPA	the hypothalamic-pituitary-adrenal axis.
PNI	psychoneuroimmunology
ROT	reality orientation therapy
SAM	sympatho-adreno-medullary axis
WHO	world health organization

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Abstract

The adoption of *One Health*, the historic resolution that ensued from the understanding between the American Medical Association and the American Veterinary Association in 2007, defined and promoted the concept of One Health as *the combined effort of multiple professional disciplines, locally, nationally and globally, to achieve optimal health of humans, animals and the environment*. Despite One Health was at first aimed at infectious diseases, over time it has inevitably permeated other areas, emphasizing how different health issues should be addressed through a holistic approach which looks at the overall health through the development and application of advanced and multidisciplinary adaptive solutions (The Manhattan Principles, 2004) whose aim is preventing epidemics and maintaining the integrity of ecosystems for the benefit of humans, domestic animals and biodiversity.

As a result of the need to evaluate the person in its complexity, in the context of human and veterinary medicine, there has been a growing interest in integrated approaches whose aim is prevention, diagnosis and treatment of certain diseases. More specifically, the need to develop new policy approaches that can also act upon environmental, psychosocial and lifestyle factors has led to the creation of *Healthcare Zooanthropology,* understood as the study, administration and application of the relationship between humans and animals in contexts that are both healthcare/therapeutic and didactic which can be an effective resource for health promotion.

In this specific context, the Doctor in Veterinary Medicine casts his figure in the social dimension as the bridge of the interspecific relationship and as the synthesis of its expression by serving as the professional guarantor for the safeguard and the promotion of health and welfare of humans and animals alike through the development of specific health and therapeutic protocols that are applicable within specific Animal-Assisted Interventions (AAIs).

This dissertation has two main objectives. The first is to apply the Federico II *Model of Zootherapy* whose multidisciplinary team is entrusted with activating a communication system and an interspecific relationship that has the dog co-therapist as its focus (Menna, 2016a). The second objective is to verify the data pertaining to the effects of three different possibilities of application of the above mentioned Model. To do so, the analyses of psychological and biochemical parameters were conducted on the humans whereas the behavioural and health parameters were tested on the animal involved. The experimental application of the Model was carried out in three different contexts connected to ageing. More specifically:

1) Evaluation of the efficacy of animal assisted therapy based on the reality orientation therapy (ROT) in Alzheimer's disease patients via analysis of psychological parameters: Mini-Mental State Examination (MMSE) and 5-item Geriatric Depression Scale (GDS).
2) Zootherapy as an wellness-promoting intervention in patients affected with Dementia and depression via salivary cortisol measurement in the Veterinary Zootherapist, co-therapist dogs and in the patient(s).
3) One project in health prevention and well-being promotion in older healthy individuals by enhancing the symbolic value of the dog and through the measurement of salivary cortisol in the Veterinary Zootherapist and in the elderly.

Moreover, in line with the One Health policies and as planned for by the specific health-related protocols fostered by the Federico II Model, three different investigations were conducted so as to correctly analyse, evaluate and prevent zoonosic risk in dogs that were involved in the AAIs settings:

1) Epidemiological survey of *Pasteurella multocida* from oral cavity of dogs.
2) Epidemiological survey of Thermotolerant *Campylobacter* in dogs at Dog Educational Centres.
3) Parasitic infections in dogs involved in Animal Assisted Interventions.

The key focus and strength of our research study revolves around the investigation conducted on the therapeutic effects of Zootherapy. Yet, we dare to say that this study is among the first at international level with regards to the process analysis of Animal-Assisted Interventions as it expresses itself through the scientific premises, the intervention protocols and through the results that were obtained by theorizing and by applying the Federico II Model of Zootherapy.

I One Health

The term *One Medicine* was coined by Calvin Schwabe (Schwabe, 1984) who is known as one of the fathers of modern Epidemiology. One Medicine became *One Health* in 2004 when Human Medicine, Veterinary Medicine and Environmental Sciences joined forces to prevent the emergence and spreading of zoonoses and thus protect human health (Canadian Public Health Association). One Health, as we shall refer to it, is an approach that views all global health threats from a cross-disciplinary collaboration and communication perspective. As such, it aims at enhancing human and animal health through an ongoing collaborative effort amongst health professions, physicians and veterinarians in particular. The historic cooperation between the American Medical Association (AMA) and the American Veterinary Medical Association (AVMA) was a crucial step of this process (2008). The use of human and animal sentinels for health hazards is very helpful in detecting and in managing shared health risks more swiftly and efficiently (Rabinowitz, 2009). Moreover, the veterinary diseases surveillance networks have a key role in human surveillance as well. In Italy, a centre of excellence in this context is the *Centro di Riferimento Regionale per l'Igiene Urbana Veterinaria della Regione Campania (*C.R.I.U.V.). The main task of the CRIUV is to monitor the state of the environment's health through data analyses of diseases in dogs living in contact with man, both in urban and in rural environment. In this georeferenced data gathering task, dogs are a useful source because they represent an authentic *sentinel* of environmental pollution. Once collected, the data serves Human Medicine by providing a detailed picture even as far as the actual prevalence and incidence of tumours and other chronic pathologies, which can be traced back to pollutant exposures in specific areas considered at risk.

In the last few years, emerging diseases have gained a leading role in epidemics on global scale, and zoonoses are dominant (Gibbs, 2009). These diseases which impact both domestic and wildlife fauna are currently the main threat globally for human health. Therefore, a synergy between human medicine and veterinary medicine is indispensable for research, surveillance and control of this global health challenge.

It is important to note that the concept of health as it pertains to humans involves all aspects. Amongst them is mental health which, through the human-animal bond phenomenon, gives pets an important role to the human well-being.

This is evidenced by Animal Assisted Interventions which assign pets with an important role in maintaining and improving human health.
As the One Health mission statement says:

"Recognizing that human health (including mental health through the human animal bond phenomenon), animal health, and ecosystem health are inextricably linked, One Health seeks to promote, improve, and defend the health and well-being of all species by enhancing cooperation and collaboration amongst physicians, veterinarians, other scientific health and environmental professionals and by promoting strengths in leadership and management to achieve these goals".

This approach represents a change in perspective even when disease control and prevention are considered. The drive for collaborative effort between human and veterinary medicine is beginning to rise. Yet it is a global voice. It would represent a new paradigm of health management with tangible results such as: a more effective and sustainable organization of public health, reducing the risk of zoonotic but most of all it would bring to the forefront that the importance of animals for human health goes well beyond zoonoses. Indeed, a collaborative effort between human and veterinary medicine would go deep into the heart of what we intend by relationships and all the complex psychophysical implications that may emerge (Cipolla, 2013).

II Veterinary Public Health

In recent decades, the concept of Public Health has undergone a significant evolution. For a long time, it had been identified with the principles of prophylaxis and the fight against communicable diseases. Currently, its broader meaning covers areas like: community health status, health promotion, health services and health care, planning, administration and management services. The result is that many disciplines work within the field of Public Health; amongst these: Epidemiology, Health Economics, Sociology, Health Policy.
According to the World Health Organization (WHO), Veterinary Public Health is that aspect of Public Health whose scope is the application of veterinary professional skills, knowledge and resources to protect and improve human health" (WHO 1999). This implies that Veterinary Public Health is not solely concerned with safeguarding the economy and providing environmental protection of areas where man and animals coexist. The scope and competences of Veterinary Public Health, as the WHO says, are extended to any and all actions meant to improve human health where animals are involved. It is our strong belief then that, in addition to Animal Production, the Environment, Biomedical Research, Emergencies and Social Aspects, Veterinary Public Health ought now to include Animal Assisted Interventions especially when these address human health improvement and optimize the human-pet relationship.

Fig. 1 Prof. S. Prosperi - Veterinary Public Health.

Health improvement, moreover, aims at well-being which is basic and intrinsic to human health, as we read in the WHO definition: "*a state of physical mental and social well-being and not merely the absence of disease*" (1948). As a result, AAI are a type of therapeutic work that must be classified as part of health services rendered just as Intervention must be accepted as operating within the field of human health. It becomes clear, therefore, that Healthcare Zooanthropology is a new field of study and a potential professional category for Veterinarians in Public Health.

III The role of the Veterinary Doctor in Public Health

Veterinarians receive extensive professional education both in medicine and in clinical science. Some of the topics that are studied include anatomy, physiology, parasitology, pathology, anatomical pathology, virology, microbiology, epidemiology, population health, infectious diseases, zoonoses, therapy and pharmacology, preventive medicine, ethology, behavioural medicine. Moreover, they deal with patients of different species and are familiar with comparative medicine.
Due to this educational training, contributions by Veterinarians to Public Health are multiple and cover several different fields such as (Cipolla, 2013):

- Institutions, Health Authority and Healthcare System.
- Clinical practice: companion animals or livestock.
- Food safety and nutrition.
- Pharmacology and drugs.
- Wildlife fauna.
- Environment.

Finally, in view of the Public Health scope, Veterinary practice involves all the aspects of human health, including mental health and the array of emotional aspects through the human-animal bond phenomenon (Lagoni, 1994). As already mentioned in this chapter, the concept of Public Health has undergone a significant evolution. Among the contributors to this deep change, a special mention goes to Dr. Adriano Mantovani, Professor of Veterinary Infectious Diseases at the University of Bologna, who did very much to provide evidence as to the importance of this needed change. Through innumerable studies, his contribution went as far as foreseeing a wider definition of zoonoses (Mantovani, 2000) which, from simple infectious animals transmitted diseases extended to any form of animal-caused damages to humans, including even non-infectious damages such as microbial drug resistance but also bites and other injuries. Mantovani's foreseeing contribution has profoundly transformed the world of Veterinary Public Health. The result in Italy has been that the responsibilities of the Veterinary Doctor have become even more ingrained in the social fibre and the Hygienist Vet has taken on the role of protector of public health, within the urban context in particular. It is also owed to Mantovani that Veterinary Public Health is viewed as part of Public

Health for the social responsibilities it oversees (1976). In Mantovani's final draft (2000), zoonoses are defined as a *"Damage to the health and quality of human life, resulting from relations with animals whether these are vertebrate or invertebrates, edible or poisonous".* This concept was reinforced and summarized by Blancou (2000) as "A risk to health and/or the quality of human life arising from relations with animals".

Further help in contrasting zoonoses came in 2011 when Hodgson and Darling introduced the concept of *Zooeyia* into One Health. This "positive inverse of zoonosis" is only one of the many efforts worldwide of an inversion process. As far as *Zooeyia*, it provides humans with multiple benefits from interacting and bonding with companion animals; as it provides "the evidence base for the philosophical construct of the human-animal bond" (Hodgson & Darling, 2011).

The human–animal bond is *"a mutually beneficial and dynamic relationship between people and animals that is influenced by behaviours that are essential to the health and well-being of both. This includes, but is not limited to, emotional, psychological, and physical interactions of people, animals, and the environment"* (Chalmers, 2015).

The strengths of this bond and the potential implications of it for human health and well-being are the fundamental premises of the AAIs which provide the Veterinary Doctor with ample education and professional placement within Public Health. In the Federico II Model the Veterinary Doctor plays an active and decisive role in the AAIs setting as handler and guarantor of the animal's well-being.

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Chapter 1

1.1 The Zooanthropology: a step in the evolution of the human-animal relationship

Zooanthropology, meant as the discipline that studies the man-animal relationship, is contributing to a shift in how man defines himself. Man's view of himself as a self-sufficient being is turning into a view of the self that defines itself by integrating otherness and by creating cultural hybrids.
Zooanthropology, owes much to Anthropology, as it helped unveil the importance of the reference animal as a peculiarity of different cultures and as a universal presence inside cultural systems. The assumption in all of the research on the significance of the animal reference is to be attributed to the analysis brought forth by Claude Lévi-Strauss on the "good to think with" animal. Zooanthropology, moreover, owes Ethology and especially Zoosemiotics and Human Ethology for their contribution to the understanding of interspecific interaction and communication systems. In its attempt to explain issues such as man's interest toward the animal and the adoption and domestication phenomena, Zooanthropology has indeed benefited from the findings in these fields as it dealt with the cultural aspects rising from human-animal interaction and with the behavioural bases of the human-animal relationship A special mention goes to Konrad Lorenz who was the first to study interspecific relations. Lorenz affirmed that the man-animal relationship ought to be interpreted according to the Interspecific Adoption Rule and the inherent universal zoosemiotic codes. So that the drive behind the whole domestication process should be researched in the parenting inspiration that puppies evoke in humans (Lorenz, 1950). Expanding on this premise, Ethologist James Serpell saw it as a Reciprocal Relationship: so that if man adopts and exploits the animal's traits, the animal evolves in its ability to stimulate parental care and thus takes advantage of the human environment (Serpell, 1986). Entomologist Wilson, father of Sociobiology, was among the first to consider this unconditional drive towards the animal (1984). According to Wilson, there is in man a *biophilia* or rather a motivational drive towards all that is alive and the animal is the instinctual drive par excellence for a human baby. Likewise, the animal seems to assume an all-encompassing role in interpreting the world around, or rather, it would immediately be credited as the mediator in knowledge acquisition. This hypothesis is supported by research on Human Ethology and in particular in studies by Eibl-Eibesfeld (1989). According to these studies, the animal is an entity with such extreme

fascination for humans that they cannot avoid its presence. For Zooanthropologists, there are two probabilities that would explain this latest trend; respectively: the *Primary* and the *Cooperative.* The *Primary* includes positions such as Lévi-Strauss' who held the animal "as good to think with" and not only to eat (1974) as well as the concept developed by Eibl-Eibesfeld, one of Konrad Lorenz' disciples. According to Eibl-Eibesfeld, amongst man's many innate traits, there is a tendency to see animals even where none are there, using the animal then as an cognitive operator. For the *Cooperative* Probability, however, the human brain undergoes a different development in the post birth period, in the sense that, compared to new born in other species, a human child is born more immature. If this causes humans to seek greater parental care, it also allows them to develop a greater capacity to provide better care. Defining for ourselves this capacity of taking care as a vocation, our tendency to adopt animals more easily, then, is due to the human condition of feeling more vulnerable to the signals that puppies of other species emit.
One theory that differs from these approaches is the so called the *Zootrophy Theory* or rather the current vocation in our species which allows and facilitates interacting with the animal. According to *Zootrophy*, in the relationship that man establishes with the animal, there is a disposition to dialogue and not to deceive. The interspecific relationship is then dimension-based and sizeable. It is based on an interchange that leads to a change through specific relationship dimensions where some of these increase exploration in the person, other favour introspection; others still, reinforce the self or enhance self-esteem through self-efficacy mechanisms, and other dimensions yet act on support.

1.1.1 Concept of the otherness *in Zooanthropology*

The human-animal interaction has been present on the evolution path throughout the ages. Scholars have defined it as having undergone three different phases (Sechi, 2012):
- *archaic phase*: the "totemic magic" relationship: the animal had all of the rights and requirements of a higher being and embodied the God in its forms (irrational phase);
- *economic-functionalist phase*: it rose along with domestication, man affirmed his superiority and rights over animals; an anthropocentric view of the universe is defined (rational phase);

- *equal rights and ethics phase*: animals, step by step, and according to species, become repositories of rights (consciousness phase).

The single phases represent the evolutionary and malleable trend of the human-animal relationship. In the culminating and third phase, it represents a new way of feeling of current society (Marchesini, 2005). It is from today's self-expressions that arise "new" needs which sometimes require animal mediation to be satisfied.

The term *otherness*, from the Latin *alter*, means "different". In philosophy, it is the opposite of *Identity*. Within the field of human-animal interaction (HAI), Zooanthropology, studies the Relationship, that particular aspect devoted to the dialogue, as it studies the Animal Reference which defines the referential role that the Pet entertains in the relationship. Animal "Otherness" is recognized then in the attributes that reflect subjectivity, diversity, peculiarity.

The ways of interacting with an animal differ: from it serving as a stimulus or a tool to its becoming a psychological projection that enhances the symbolic aspect that represents it. Yet despite this, man still remains the referent subject within an anthropocentric perspective. Instead if we are referring to an actual relationship with the animal, we can not dispense from recognizing the otherness involved in a mutual dialogue and so a reciprocal communication which leads to a defining of each other. In this manner, the animal shares in the role of reference subject.

According to this approach, the animal becomes a peculiar subject which is recognized with its own ability to communicate, all to be learned and interpreted. This is how it is recognized as having an active role and an exchange capacity (Menna, 2016a).

Conceiving all of this, and most of all "embodying" this concept, requires quite a remarkable shift in how the world is interpreted. It will take abandoning the anthropocentric perspective and making a deep cultural transformation that leads to an epistemological change and the adoption of a systemic type of thinking. To date, the Federico II *Model of Zootherapy* fully embraces this vision.

1.2 The interspecific human-dog relationship

Dogs [*Canis lupus familiaris*] are considered the first animal species that humans domesticated. Genetic and archaeological evidence suggests that this process began approximately 11-16kya (Freedman, 2014; Davis, 1978). Dogs and wolves [Canis lupus], their closest living relatives, differ in a variety of phenotypic traits, despite only differing in ~0.04 7 % of nuclear coding-DNA sequence (Wayne, 2007). Particular attention has been given to their behavioural differences, with dogs showing a greater ability to read human communicative behaviour (Hare, 2005). When and how these new cognitive abilities emerged remains unclear. It has been suggested that rather than selection for these specific behaviours it was selection for tameness, a reduction in fear and aggression towards humans, that permitted dogs to express these latent abilities, which in wolves are inhibited due to their fear response (Hare, 2005, Range, 2015).Unlike the majority of domestic species, which were primarily selected for production related traits, dogs were typically selected for their behaviours (Serpell, 2014). Throughout the era of domestication, dogs have been selected for traits that enhance their sensitivity to a wide range of human communicative signals, both visual and acoustic (Miklosi, 2009). Several recent books (Bradshaw, 2014; Kaminski&Marshall-Pescini, 2014) have pointed to dogs having developed specific socio-cognitive capacities to communicate and form relationships with humans. Most likely due to a mixture of factors, phylogenetic (domestication) and ontogenetic (experience), dogs sharing the human household demonstrate high levels of attentiveness towards human behavior (Virányi, 2008); are the best amongst animals in following human gestures, (Kirchhofer, 2012); show a high sensitivity to human ostensive signals such as eye contact, name calling and specific intonation (Topál, 2014) and exhibit an increased readiness to look at the human face (Gácsi, 2004). By monitoring human faces, dogs seem to obtain a continuous stream of social information that range from communicative gestures to emotional and attentive states (Schwab & Huber, 2006). Even if this does not imply that dogs can read the human mind, it does mean that they are highly discriminating in reading our behavior. This so-called emotional evolution placed dogs in a new adaptive space in which they were able to interact with humans as comfortably as with their conspecifics (Hare, 2005), and this laid a foundation for the establishment of the human-dog bond.
There are still very few empirical studies that concentrate on the specific na-

ture and the processes governing the human-dog bond focusing on the whole relationship, and not solely on the human side (Topal, 1998). Among the different types of social relationships, the Ethological Model (Bowlby, 1978; Ainsworth, 1972) considers the human-dog attachment from a biological and evolutionary perspective, emphasizing the adaptive function. In the Ethological point of view, attachment is a particular type of lasting affective bond that a person or animal establishes with another being through lifetime (Ainsworth & Bell, 1970; Cohen, 1974). For some scholars, the human-dog relationship has much in common with the parent-child relationship (Askew, 1996). The Ethological Attachment Theory along with the "Strange Situation" Procedure were initially developed to investigate the bond that gets created in the human species between parent and child, mother and child in particular (Ainsworth & Wittig, 1969; Ainsworth, 1978). However, attachment and its related behaviours were also studied in a comparative perspective in other social species. In particular, some research has used the Strange Situation Procedure to analyse the emotional ties and attachment in relations between people and beings belonging to a different species (Bard, 1991; Topal, 1998). The most recent research on the human-dog relationship focused on the emotional bond between the two beings and the role that the animal plays in the human's psycho-physical well-being (Serpell, 1995; Menanche, 1998; Wilson & Turner, 1998). A recent study that investigated the relationship dynamics among a dog, his owner and a stranger (Prato Previde, 2003) showed that in a Strange Situation setting whereas an adult dogs with his master has behavioural changes that reflect intense bonding characteristics, with the stranger in the presence of the owner, these were only indicative of a secure base effect with regards to social play. These findings have led us to reflect on the dynamics of the relationship within a Zootherapy setting. In the settings we have worked in, the dog-owner-stranger triad is replaced by the co-therapist dog/the Veterinary pet handler and the patient. Among them, through the *game,* different relationship/interaction modes progressively develop that implement the typical features of an attachment bond. These features include: a sense of security; comfort in the presence of others, and using the partner (Vet) as a "secure base" (Ainsworth, 1989).

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Chapter 2

2.1 Animal assisted interventions (AAIs): characteristics and classification

The Anglo-Saxon term *Pet Therapy*, or rather *Therapy with an affectionate animal*, was coined by Child Psychiatrist Boris Levinson who by chance observed that having his dog Jingles present was improving his sessions with a young autistic patient. Levinson realized that, in the dog's presence, the child was more willing to start a conversation with him just as he observed that the dog could serve as a relational and interspecific bridge. He included these remarks in his 1961 book "The Dog as Co-Therapist".
In our days, the term Pet Therapy has given way to the AAIs acronym which stands for Animal Assisted Interventions when these are meant to be of benefit, be it therapeutic-rehabilitation, recreational and/or educational.
Depending on the activity context, AAIs are classified in:

- Animal Assisted Therapy (AAT) are therapeutic rehabilitation interventions aimed at the care of disorders related to the spheres of: neuromotor, psychomotor physical, cognitive, emotional and relational; such interventions are patient-tailored per pathology.

- Animal Assisted Activities (AAA) refer to interventions that have leisure and recreational purposes, meant to improve the person's quality of life through appropriate interaction with the animal involved.

- Animal Assisted Education (AAE) refers to educational interventions which are designed to promote, enable and sustain the individual's resources and potentials through the animal's mediation.

The ways humans bond with pets and how this is externalized has led to pets being extensively employed in different settings with the implication that this kind of bond is among the elements that contribute to patients' therapeutic gains (Friedmann, 2009). Moreover, though true for animals in general, dogs' affectionate response to attentions from humans and their eliciting pro-social behaviours and positive feelings defines their unique capacity that may serve as an emotional bridge in interaction mediation in therapeutic contexts (Serpell, 1996).
As reported below:

Animal-Assisted Therapies (**AAT**) are goal-driven interventions of which a specifically trained animal is an integral part of the treatment process. AAT are designed to foster betterments in human physical, social, emotional, and/or cognitive functioning. They are provided in a variety of settings and involve the individual. Documentation and evaluation throughout this process is essential.

Animal-Assisted Activities (**AAA**) Provide opportunities for motivational, and/or recreational benefits to enhance quality of life in humans. AAA are delivered in a variety of contexts in cooperation with animals that meet specific safety criteria.

Animal-Assisted Education (**AAE**) Relies on use of animals' presence for specific educational purposes, as in cases involving children.

2.2 Legislative references as pertaining to AAIs

At present, in Italy AAs have been recognized as official care by a Legislative Decree (DL.vo issued on February 28th, 2003), however there is no specific legislation regulating them. This poses serious problems in terms of safety and protection of humans. Indeed, those who can benefit from AAI are frail subjects and are mainly represented by children as they are often extremely trusting and may easily achieve a good level of intimacy with animals, as well as elderly persons. In AAI, the activity performed by the "animal therapist" towards the "human patient" is very complex and to be successful, above all else, it should entail the contribution of many professional figures. For this reason, a combined effort of a cross-disciplinary team made up of various professional categories should always characterize an AAI program. In order to promote research, standardize operative protocols and boost cooperation between Human and Veterinary Medicine, the Italian Ministry of Health along with representatives from Regions and Provinces, in March 2015, approved the Agreement entitled "Linee guida nazionali per gli interventi assistiti con gli animali (IAA)" [National guidelines for Animal-Assisted Interventions (AAIs)]. The scopes of this Agreement are:

- defining operational standards for the correct and appropriate application of AAIs on national territory.

- singling out tasks and responsibilities for the professional figures and the operators involved.

- defining educational training modes for the professional figures and for the operators involved.

2.3 AAT as a new therapeutic support unit

Specific human pathologies such as Alzheimer's, Alexithimia, Parkinson's, Autism, stress-related mental disorders and so on, have led research to discover and evaluate non pharmaceutical rehabilitation strategies (Cukor, 2009; Bomyea & Lang, 2012). Previous studies had indicated increased quality of life and self-rated health for patients that receive a combination of conventional and complementary therapies, i.e. integrative care. The objective is to provide patient-centred, personalised, safe and effective health care with as few side effects as possible (Sundberg, 2015). In view of the positive effects demonstrated and documented with respect to the therapeutic involvement of pets (Beetz, 2012), the Animal-Assisted therapies can be considered as Medicine Intervention Co-therapies in support to conventional therapies.

2.3.1 Effects of AAIs: literature review

Effects on social interaction

Human-animal interaction plays a role in human-to-human social interaction. It stimulates social behaviour and increases receiving positive social attention from others. Likewise, this type of influence involves trust, empathy and positive mood. In and of itself, interaction with an animal is a form of social behaviour and different studies focused on its "social catalyst effect" or rather how the presence of an animal facilitates interpersonal interaction (Wells, 2004; Kotrschal & Ortbauer, 2003). Children with autism, for example, showed having the highest and longest interaction frequency with a real dog than with an object or a person (Prothmann, 2009). Similarly, when children with pervasive developmental disorders (including autism) were interacting with a dog, they showed greater playfulness than they did with toys just as they were more aware of their social environment (Martin & Farnum, 2002). Many AAIs focus on elderly residents or patients. The presence of an animal had a positive influence on social interaction in elderly psychiatric inpatients (Menna, 2012; Haughie, 1992). When AAT were compared to non-AAT recreational sessions in long-term care facilities, the animal involvement was shown as linked to conversations being started more frequently and lasting longer (Bernstein, 2000). From their reviews on the effects of animals-assisted therapy on patients with dementia, Filan & Llewellyn-Jones (2006) and Perkins et al. (2008) concluded that AAT can benefit these patients by increasing social behaviour and interaction.

Reduction of depression and promotion of a positive mood

Souter and Miller (2007) in their meta-analysis and Menna (2016b) conclude that AAI may significantly reduce depressive symptoms. In two controlled studies of patients in long-term care facilities, Banks and Banks (2002) showed that animal visitation programs reduced feelings of loneliness. Likewise, animal contact is known to improve mood in children and adults experiencing physical or mental health problems. Nathans-Barel et al. (2005) compared an AAT group and a non-AAT group in a 10-week long program for patients with chronic schizophrenia and found that the AAT patients' mood improved. Children with psychiatric disorders showed better intra-emotional balance after only a single therapy session with a dog. In hospitalized children, mood improved with AAT and with traditional play therapy as the parents and children themselves reported; yet, displaying positive feelings was only associated with AAT.

Effects on cortisol, epinephrine, and norepinephrine

HAI has been investigated for its effects on hormonal indicators of stress such as cortisol, and on neurotransmitters such as epinephrine and norepinephrine. These studies provide direct evidence that interaction with a pet, in particular a dog, positively affects endocrine responses as indicated by changes in the levels of cortisol, epinephrine and norepinephrine, thus suggesting an attenuation of stress responses via HAI. Barker et al. (2005) compared the effects of 20 minutes of quiet rest to 5 and 20 minutes interaction with a therapy dog in healthcare professionals. A significant reduction of serum and salivary cortisol, but no effects on the other parameters, were found in the dog conditions. A study by Cole et al. (2007) compared a visit with a dog to a visit without a dog and the usual care in the hospital as control conditions among adults hospitalized with heart failure. Significantly lower epinephrine and norepinephrine levels were measured during and after the dog visits. Beetz et al. (2011) investigated the effect of social support by a dog compared with support by a friendly human during a social stress test on the cortisol levels of children with insecure attachment representations. The support by a friendly dog during the experiment was associated with significantly lower cortisol levels than support by a friendly human. This effect was strongly correlated with the time the children spent in physical contact with the dog during the experiment.

Effects on blood pressure, heart rate, and heart rate variability

A substantial number of studies investigated the effect of HAI on blood pressure and heart rate, some also included skin temperature or skin conductance, either in the absence of a specific stressor or during a stress-inducing task. A positive attitude toward pets was associated with lower mean arterial pressure and systolic blood pressure. Vormbrock and Grossberg (1988) assessed heart rate and blood pressure while undergraduates visually, verbally, or tactually interacted with a dog. Blood pressure was highest while talking to the experimenter and lowest during stroking the dog. In adults hospitalized with heart failure, a 12-min visit by a person with a dog led to a greater decrease in systolic pulmonary artery pressure during and after the visit when compared to a visit by a person alone (Cole, 2007). Motooka et al. (2006) employed heart rate variability as a parameter associated with autonomic nervous system arousal in healthy elderly adults walking with or without an unfamiliar dog for 30 min. While walking the dog, heart rate variability was significantly higher than when walking alone. Handlin et al. (2011) showed that stroking ones' own dog for just 3 min led to decreased heart rates 55 min later in female dog owners, while no such response was observed in a control group not petting a dog.

The studies that follow assessed the effect of interactions with unfamiliar animals on heart rate and blood pressure before, during or after a stressor. Nagengast et al. (1997) found heart rate and systolic blood pressure of 3–6 year-old children during a standardized physical examination as a mild natural stressor to decrease more in the company of a friendly dog than when undergoing this examination alone at another time. Overall, most of these studies show that the presence of friendly animals, both familiar or unfamiliar, can effectively reduce heart rate and blood pressure or buffer increases in these parameters in anticipation of a stressor.

Reduction of fearand, anxiety and promotion of calmness

Several studies investigated whether animal contact can reduce fear and anxiety elicited by a stressor. Shiloh et al. (2003), Barker et al. (2003), Cole et al. (2007) found that anxiety was reduced most in the presence of the visiting dog when they compared the effects of a 12 minute visit with a dog or without a dog, with conditions of normal care in adult patients who had been hospitalized due to heart failure. In a study by Lang et al. (2010) patients with acute schizophrenia reported less anxiety after a clinical interview where a

friendly dog was present than when it was conducted without it. Contrary to a nursing home without a dog, in one that had a resident dog, elderly residents reported less tension and confusion.

Effects on the immune system

Charnetski et al. (2004) reported a significant increase in salivary immunoglobulin A (IgA), an indicator of good immune system functioning, in college students after stroking a live dog in comparison to stroking a stuffed dog or sitting quietly for 18 min.

Effects on learning

In a series of studies Gee and colleagues researched the effect of the presence of a dog on children performing different tasks. A group of language-impaired and a group of typical preschoolers developmentally delayed preschoolers and a group of normally developed children performed faster in a motor skill task with the same accuracy when a dog was present than when it was not (Gee, 2007). The increase in performance speed, the authors argued, might have originated in the dog serving as an effective motivator just as its presence might have led to increased relaxation and a reduction of stress during execution of the task. In another study the presence of a dog, rather than that of a toy dog or of a person, showed better results when pre-school children with and without language impairments adhered to instructions during an imitation task (Gee, 2009). This consisted in needing fewer prompts (as an indicator of concentration) for a memory task in the presence of a dog contrary to their needing the most prompts when another human was present (Gee, 2010b). Furthermore, in a match-to-sample task, pre-school children made fewer errors, such as irrelevant choices, in the presence of a friendly dog in comparison to the presence of a stuffed toy dog or a human (Gee, 2010a).

Currently, there is no direct evidence that animals can promote learning in humans, but the presence of a dog in an educational setting seems to support concentration, attention, motivation, and relaxation which reflect reduction of high stress levels that inhibit effective learning and performance (Beetz, 2012).

Summary of effects of AAIs:

- improvement of social attention, behaviour,interpersonal interaction, and mood
- reduction of stress-related parameters such as cortisol, heart rate, and blood pressure
- reduction of self-reported fear and anxiety
- improvement of mental and physical health, especially cardiovascular health
- improvement of immune system functioning
- reduced aggression
- enhanced empathy
- improved learning

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Chapter 3

3.1 The Federico II Model of Zootherapy

The main characteristic of this Dissertation is the experimental application of the Federico II Model of Zootherapy (Menna, 2016a) within the AAIs. As seen earlier, the nature and quality of the human-animal interaction was and continues to be the topic of extensive literature, especially with regard to the "therapeutic" effects that the animal has on man. According to this Model, and in contrast to conventional scientific methods which are intent on "reducing" everything to the observable reality, the cornerstone of the therapeutic success, in full respect of health for human and animal alike, is due to a new epistemology that is able to encode interspecific communication processes based on perception and on respect for diversity among all involved. In summary, the Federico II Model of Zootherapy aims at developing the AAIs, not as a pure therapeutic technique based on one-way communication, but as complex interventions in which the different species involved are put into play by participating in a dynamic process of change. By bringing together in a homogeneous reflection the various experiences and ideas gained over the years from fields such as Biology, Anthropology and Psychology and in line with Bateson's thinking as opposed to the Cartesian Dualistic Model (Iacono, 1990), the Federico II Model has been based on the recognition of Otherness without which there is no access to the possibility of communication and to the idea of a "System".

The word relationship derives from the Latin verb *religare* and it means "to tie together". In this action, the drive of living beings to create a connection with each other is as present as their becoming "bound together". The word Relationship has at its core then the concept of reciprocity. To define a relationship in its characteristics, certain parameters need to be present which allow identifying it. Amongst these, we note: the content of the interaction; its quality; its frequency; the identity of those involved; their motivations; their ends and means; and the roles they respectively hold. Just as in any kind of relationship, these are also found in the therapeutic relationship which includes the interspecific relationship, the basis of Zootherapy. This rationale has led the Department of Veterinary Medicine and Animal Production of the University Federico II of Naples to introduce a Multidisciplinary Team Model. This team, made up solely by professionals, is meant to provide therapy and show team cooperation by interacting and integrating knowledge from their respective fields.

The ability to create a therapeutic relationship through interspecific communication is the roots of therapeutic success in a Zootherapy setting. Communication is an interactive exchange between two or more participants and has to be thought as "a circular process, within which the behaviour of each and every member influences and is influenced that of all others". It should also be remembered that in communication, spoken or written language is only but a small part of the mutual exchange. The rest is expressed non-verbally (proxemics, facial expressions as well as any attitude that our body takes on) then: "We cannot not communicate" (Watzlawick, 1971). This vital aspect can in no way be underestimated when an animal is involved in a therapeutic activity since its communication mean is non-verbal. It is precisely by keeping away from verbal communication that in an interspecific communication the phenomenon of empathy gets triggered or rather the ability of comprehending what the other is feeling as well as the emotional context immediately. This in therapy is crucial in favouring an atmosphere of understanding, warmth and taking part by all involved. Consequently, the animal, the relationship established with it and the very same therapist-animal relationship create the premise for patients to freely express emotions and feelings, fears and anxieties while they favour tolerance of the self and understanding for one's resources and limits.

It is clear, then, that amongst the styles used for AAIs, the Federico II Model of Zootherapy primarily distinguishes itself for how it conceives the presence of an animal. For our Model, the animal is an Active Referent. On one hand, it has specific competences in interpreting non-verbal language and in serving as an emotional resonance of the person. On the other hand, through its own behavioural language, the animal provides key messages to the human relationship expert. Once these messages are properly interpreted and applied, they lead to therapeutic results. Zootherapy implies an interspecific relationship aimed at care, in which all beings involved in the therapeutic system represent a team, a system in which every one is aware of its functions, resources and expertise and, above all else, of its responsibility. In the Federico II Model, the animal is not viewed as a passive object but rather as a co-therapist and, thus, as an active member of the setting. This is made possible thanks to its innate ability to express itself which, through education, can be refined and strengthened to contextualize a situation. We hold this to be the only way to achieve real recognition of the reciprocal diversities and full respect of the animal's own specificity.

3.1.1 The Zootherapy Team: a system that provides care

Similar to all other therapeutic relationship activities, Zootherapy is a process. In these activities, the therapeutic function gets expressed throughout the course of the relationship just as, during its unfolding, there are occurrences of empathetic and transfer phenomena. If these represent the relation's strength and power, they also characterize its enormous complexity. In an interspecific system, it is important that the animal be recognized in its otherness, in its specificity, in its uniqueness, acknowledging it not only as a useful tool but as a competent individual. The wealth of an interspecific therapy system lies in what the animal gives in feedback to its tutor (Veterinary as handler) in both emotional and behavioural terms and how this information can become therapeutic experiences.

3.1.2 Team structure

According to the Federico II Zootherapy Model, the team is made up of a Veterinary Zootherapist, the co-therapist and thepsychologist/psychotherapist who works as the Interspecific Relationship Expert (IRE) (figure 3.1). The rigour in choosing these professionals, who are to provide therapies, is linked to their university training, which is the mastermind of an indispensable mindset in configuring a therapeutic work plan.
More specifically:
The **Psychotherapist Interspecific Relationships Expert (IRE)** is entrusted with the interspecific relationship in all its aspects. Thanks to his interdisciplinary training, he has the appropriate tools to recognize the types and the dynamics of an interspecific relationship. In the project, he is to choose the Veterinarian and dog pair and define the most appropriate steps to undertake as the conditions and characteristics of patients in treatment require. Likewise, as the expert on human relationships, he is to register the prevalent aspects in the communicative exchange and steer the Veterinary activities as need be and based on what emerges from the Zootherapy sessions. Furthermore, he is to monitor the activities and co-operate with the Vet in structuring the setting.
The **Veterinary Zootherapist** is the animal's tutor as well as its hendler within the setting. He is responsible for the dog's health and warrants for the interspecific relationship since he handles the safety of the animal's activities

with regards to human (Mantovani, 2000). Along with the Psychotherapist, he structures the setting. Likewise, with the dog in mind, he plans a "structured game" meant to achieve the therapeutic objective as specified by the team's Psychotherapist (Menna, 2016a).

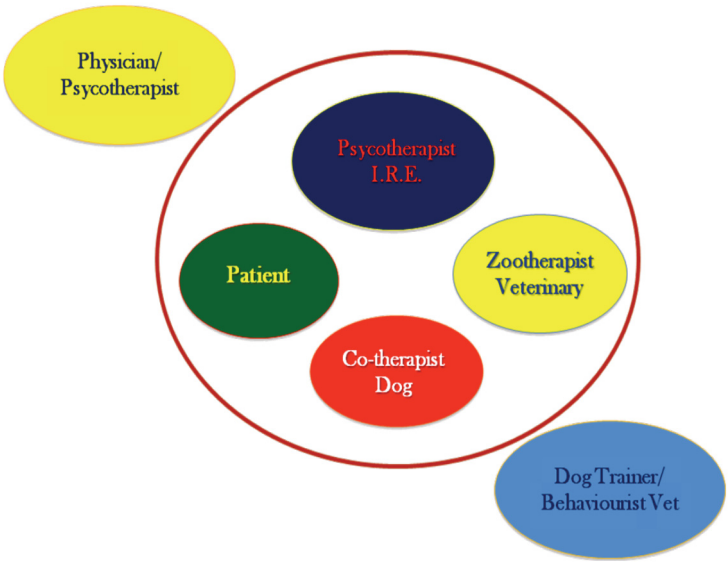

Fig. 3.1 Zootherapy team.

3.1.3 Reasons for the Veterinary Zootherapist as the animal's handler in therapies

The Zootherapist Veterinary Doctor is a professional figure within the category of Operators of the Veterinary Public Health; based on this, we emphasize the importance of health and safety within the settings. Likewise, we hold that the animal be entrusted to a professional figure subject to a code of ethics, someone in charge of patient protection from hazards of any sort due to animal activity.

Code of Ethics of the Veterinary Doctor, Article 1:

The Veterinary Doctor performs his professional business at the service of the community and to protect the health of animals and humans. In particular, he dedicates his work to:

- *the protection of people from danger and harm resulting to him from the environment where animals live, from animal diseases and consumption of foodstuffs or other products of animal origin;*
- *the prevention, diagnosis and treatment of diseases in animals and to their welfare;*
- *the conservation and functional development of livestock;*
- *the conservation and preservation of fauna based on the principles of protection of biodiversity, the environment and co-existence where compatible with man;*
- *the activities related to the lives of pets, ranging from sports competition to exotic;*
- *the promotion of respect for animals and their welfare as sentient beings;*
- *the promotion of health and education prevention campaigns for a proper relationship between humans and animals;*
- *to activities related to food production, to their proper management and the assessment of the risks involved.*

Moreover, in Italy, the recent amendments to the Criminal Code on animal mistreatment (Law #189 dated July 20th, 2004 and Article 544 in particular) assigns to the Veterinary Doctor a significant role in legal implications of any kind that might rise from activities involving animals. In 2010, a Supreme Court Sentence (9037/2010) was issued for owners' responsibility in a case where damage was caused to third parties despite the dog being on a leash. The sentence however fails to mention about any sort of responsibility in instances where dogs are involved in care activities and there is close contact work performed with people suffering from mental and physical discomforts

whose relapse -due to damage- could potentially increase in severity. Indeed, this Sentence concerned the private sector whereas therapeutic activities are a specific area solely open to professionally trained personnel inasmuch as civil or criminal liability are concerned during the project. In addition, acknowledging the compulsory presence of the Veterinary Doctor as the animal's conductor, guarding its health and thus for legal purposes being the solely responsible for it, really implies recognizing the dog's otherness. In addition, acknowledging the compulsory presence of the Veterinary Doctor as the animal's handler, safeguarding its health and thus for legal purposes being the solely responsible for it. Based on these reasons, the Federico II Model holds this properly educated, professional figure as the solely suitable to carry out therapy with an animal.

3.2 The animal in Zootherapy: the dog co-therapist

The choice of the dog to involve is perhaps the most important and most delicate in all interventions. We know from studies on the collective unconscious that the animal is a powerful emotional catalyst because it represents an archetype that every human being carries within himself (Jung, 1969; Hillman, 1985). There are two implications that arise when an animal is introduced to bridge the relationship in the Zootherapy context. On one hand, it implies it becoming "used" as a symbol at play within the patient; while, on the other hand, as a living being, it moves and communicates, giving us feedback messages. We know that non-verbal communication prevails in the animal whereas the verbal is reduced to very little. Its body signal are very swift and quite complex. When great attention is paid to them, we ensure ourselves with a powerful and valuable ally which reveals "emotional movements" as they are happening. By observing how the patient relates to the pet, we can also gain useful information on the type, quality and nature of his ties. We should keep in mind that, in essence, what is brought into the setting, what is used for the care process and defines a patient's psycho-physical improvement is nothing other than an emotional relationship in an atmosphere of trusting exchange and of reciprocity. When there is great generosity in the animal's feedback with the tutor, through a mirroring effect, the patient accepts and assimilates this gesture as confidence and self-esteem within a real relationship of reciprocity, where the exchange truly is bidirectional and, as such, the animal's otherness is achieved and acknowledged. Considered the above, the Federico II Model of Zootherapy cannot be structured without involving the dog in the therapeutic setting. Interestingly, while most studies have focused on demonstrating empathic-like responses between conspecifics (chimpanzees, rooks, elephants, rats), the domestic dog has been targeted to investigate empathic responses to humans. Due to the process of domestication, dogs have been suggested to have a heightened capacity to develop strong social-bonds with humans and a good understanding of human communication, which might have enhanced their propensity to recognize and be sensitive to human's emotions. Indeed it has been shown that dogs are capable of discriminating between human's facial expressions of negative or positive emotions. Furthermore, dogs have been suggested to show sympathetic concern towards a distressed human (Custance&Mayer, 2012). More precisely, in the latter study, dogs showed more comfort-offering behavior,

such as approaching and touching, to a human when she was crying, compared to when the human was humming or talking. Moreover, similarly to humans, it has been found that dogs' cortisol levels increased when they were exposed to infant crying but not when exposed to infant babbling and white noise (Yong&Ruffman, 2014) which is in line with the definition of emotional contagion (Quervel-Chaumette, 2016). Moreover, it is known that the dogs are able to understand referential gestures such as watching, pointing with a finger, pointing with the head (Hare, 2010; Miklosi, 1998; Miklosi & Soproni 2006; Soproni, 2001) just as they can use their ability in watching as a communication tool to locate a specific object out of their reach (Miklosi, 2000; 2003; 2004). Moreover, by monitoring human faces, dogs seem to obtain a continuous stream of social information that range from communicative gestures to emotional and attentive states. Even if this does not imply that dogs can read the human mind, it does mean that they are highly discriminating in reading our behaviour.

3.2.1 Criteria selection of the co-therapist dog

The choice in dog that will work alongside Zootherapist and patient must always depend on the special talents it has as well as its emotional and behavioural skills, all of which are required to attain the goals set. The priority is rather given to the "relational competence" of the dog who sometimes represents a real talent of the individual subject, which can be evaluated in its intra and interspecific expansiveness, its emotional stability, self-control, prudence, the trust it has "in itself" which it even expresses through its behaviour in its intraspecific group. According to the Federico II Model, it is fundamental that the Veterinarian Zootherapist and the dog share a close relationship built on empathy and mutual trust so that the Veterinarian knows the dog deeply and is able to predict it. In addition, the Veterinary Zootherapist, instead, should identify the subject that best suits the therapeutic and environmental work context and evaluate it from both a health and a behavioural point of view.
Choosing the dog, then, and training it are extremely important phases that require the cooperation of the dog trainer specialized in AAIs and the Veterinary Behaviourist, two fundamental professional figures who do side by side work.
The dog trainer, who is the most adequate professional figure in rating animals' suitability and fitness on the basis of their normal relation dynamics,

specifies the most appropriate approach modes through which a profound relationship can be welded with the Veterinary Zootherapist. He trains the dog and the pair alike to get relations that are balanced and satisfactory.

The Veterinary Behaviourist is a professional figure that differs from that of his fellow Zootherapist because he concentrates solely on the dog's behaviour dynamics. As such, he is the professional who assists the dog trainer in selecting the individual animal to make sure that no behavioural disorders are present. It is important that these two professionals serve as reference points for matters regarding the dog's "normal" and "pathological" relationship dynamics and they should be consulted whenever the Veterinary Zootherapist deems it necessary (National guidelines for AAIs, 2015).

Though there is still show a gap with respect to the standardization of criteria for the co-therapist dog selection, according to the guidelines, the animal involved should have acquired a experiential educational level that is appropriate to the degree of complexity that the project requires. Very often, the preliminary selection of the dog is still too subjective, as it refers to parameters that are not geared exclusively towards the relationship and with no scientific reference parameters regarding the interspecific therapy relationship (Wilson & Turner, 1998; Sheppard, 2002).

Normally, this refers to the minimum standards that a dog must possess (physical and behavioural characteristics) and that are provided by the Delta Society (2002) a "good dog" must actively stimulate interacting with humans, willingly accept and tolerate the reactions and behaviour of different people (Piva, 2008). Moreover, as the Delta Society states, the dog should accept touching and hugging but by the same token it should accept the most abrupt movements of the person with whom it is entertaining a relationship. Attempting to get away from the person is not a desirable behaviour during therapy, just as it isn't jumping or having contact with the patient until it is allowed. Therefore, peaceful attitude and lack of anxiety or stress-related symptoms, just as tail wagging and looking at the patient are considered the most frequently encountered behaviours and required as indices of a high level of interspecific interaction. Secondly, dogs must demonstrate a behaviour that is reliable, controllable, predictable and that inspires confidence in people/patients. At the same time, they must be able to accept being handled in a little clumsy way at times; they must be able to tolerate uncontrolled vocalizations by strangers as well as keep their attention on the people they are

working with. Furthermore, a dog should be at least one year old, to be mature enough to perform the tasks assigned to it; it should have some experience in interacting with people.
In addition to these specific social behaviours, according to the "Standard Operating Procedures", in order to achieve a correct interaction with the person, the animal must be able to provide other primary characteristics. These are:

1. the absence of any disease or medical condition that can cause a damage to both the animal and the person;
2. reliability, in the sense that the dog's behaviour should be pretty much the same in situations that are similar (and this aspect can be improved with training);
3. predictability: or, under specific circumstances, the animal's behaviour should always be taken into account;
4. suitability: namely, that the dog needs to be adequate or qualified for a purpose, which in this case is represented by the specific objectives drawn up;
5. ability to inspire trust and confidence, or rather, people involved in the interventions should feel comfortable, not threatened, serene.

For the dog to be in a state of mental and physical well-being, it must have good adaptability which is the result of a correct cognitive-relational development. A non-adapted or non-adaptable dog is one in a state of permanent stress that is related to both its inability to live a successful relationship and its frustration at not having the appropriate responses to various situations. In order for the dog to experience the latter, the relationship has to follow the parameters of suitability just as it must be fair, conscious, responsible and balanced. The animal must be recognized in its basic features, namely its attributes, and man must be available to accommodate what the animal can give him.
In this phase, moreover, we must necessarily take into consideration that learning is fundamental to the evolution process of the animal species; without learning, there is no adaptation; and, without adaptation, there is no chance of serviva (Mecacci, 2001).
Given these assumptions, for the dog to fully perform its co-therapist role in a total state of well-being and health requires that it has been involved in a

training process that can satisfy its basic mental needs. The safety of the dog depends on its being suitably introduced within social systems. This is why, during its development age, the dog must go through all of the steps that give it proper socialization, both primary and secondary, with a clear social positioning, and ability to behave properly within a relationship. Motivation represents the tendency that the animal has in the world (what it expects and what it looks for) which may be the main source of gratification or frustration. Whenever the dog performs a motivation-driven behaviour, this will be marked with a positive emotion and it will have a tendency to repeat it.
According to the Federico II Model, dogs undertake a path that enables their self-expression through an educational program as defined by the guidelines set by the Italian National Educational Sports Centre (CSEN). This is useful for the dog's learning to express itself (Gosling, 2003) and by contextualizing the situation, this aspect becomes valuable for therapeutic purposes. In the therapeutic relationship, in fact, the dog is our ally only if it is given the chance to express itself through its behaviour, in such a way as to give an emotional feedback of the setting. In addition, it is essential that the Veterinary Zootherapist and the dog entertain a close empathic and mutually trusting relationship that allows the Veterinarian to know the dog deeply and can predict its reactions which Naderi defined as cooperative behaviour (2001). This is why the dog's training process is closely linked to preparing the couple that will be involved in the therapy. Indeed, the human and relational factors can contribute to the dog's behavior and the training outcomes since they are likely to influence the dogs' affective, or emotional, states and thereby influence their behavioral output (Payne, 2015).

3.2.2 Health certification for the dog co-therapist

The Federico II Model foresees that, for every Animal-Assisted Intervention, the Veterinary Doctor solely is responsible for the health, behaviour, and welfare of the animal(s) involved in these programs. Moreover, each Zootherapy Intervention should be designed to provide reasonable assurance that animals across the spectrum of the AAI services are healthy (in part to reduce the bidirectional risk of zoonotics transmission).
A wellness program must include regular veterinary care but it goes beyond annual physical examinations and associated vaccinations and medications. The animals should be periodically monitored by the veterinarian to develop

a care continuum which will help ensure continued health and welfare.
It is important that the dog, duly chosen and trained, get its physical health and behaviour certified. It is to be done by a Public Sector Body with Veterinary Expertise which shall release an annually renewable Certificate of suitability and fitness. The dog must have a Health Certificate which includes:

1. personal data, animal identification number on microchip and enrolment in the canine registry;
2. clinical exam and previous medical history;
3. skin check for fungus and mites;
4. control of ectoparasites through pesticides;
5. control of endoparasites through coprologic examination and use of broad-spectrum wormers;
6. check for Distemper, Hepatitis and Gastroenteritis and Leptospirosis vaccinations; Rabies vaccination is only checked if the dog is employed in areas at risk;
7. check for the yearly Leishmania antibody titers; and, for Heartworm, if the dog is used in dangerous areas;
8. behavioural dog visit, run at least quarterly both during and after Zootherapy sessions as well as whenever the Zootherapist deems it necessary.
9. support from specific health protocols to contrast any pathogens with zoonotic characteristics in case it ought to come in contact with a medium-high risk setting (reception areas in medical structures, rehabilitation centres, some hospital wards).

The dog's physical and behavioural health checks should be scheduled to be periodical, from a minimum of three to six times yearly. Their responsibility falls upon the Veterinary Zootherapist and they are to be done according to the most appropriate health protocols.

Moreover, according to Federico II Model, the co-therapist dogs involved in Zootherapy Interventions are monitored through a specific health protocol with respect to any zoonoses-related pathogens such as:

- *Pasteurella* spp
- Thermotolerant *Campylobacter*
- *Giardia duodenalis* and other endoparasites

In Zootherapy Interventions, the application of these protocols is needed now more than ever as it applies also to people at high risk of infections; they are:

- children aged <5 years
- persons with waning immunity (i.e., older adults)
- pregnant women
- individuals who are immunocompromised
  (i.e., persons with human immunodeficiency virus/acquired immunodeficiency syndrome, without a functioning spleen, or receiving immunosuppressive therapy).

3.3 Zootherapy intervention protocol

Each Zootherapy session is expected to last about an hour, of which 20 minutes are to be of actual work with the dog. It is necessary nonetheless that the session always end with an emotions-related feedback as well as with the ritual washing of the hands which formally bring the session to a close.

Working methodology
The Model of Therapeutic Approach mediated by animals revolves around the concept of relationship as reciprocity. As such, it must direct its focus on Otherness, not only towards the dog which is recognized as having a competence of its own but also towards the patient who is acknowledged as a carrier of a personal history and talent. Thus, the main task of the therapists is to gather the patient's resource and ally with it so that every intervention becomes "tailored" for the person in his/her entirety and for his/her physical, psychological or psychophysical rehabilitation. In choosing and planning activities that are meant to meet specific objectives, the singular characteristics of each person have always to be borne in mind. These foster the onset of an empathic relationship and inspire the patient to undergo an "active change". It is essential that there is a reciprocal participation within this change since reciprocity stimulates improving cognitive as well as emotional functions (Menna, 2016a).

Preliminary stage
The preliminary stage of any intervention is agreed with the Medical Staff of the Host Organization which outlines their needs and rationale for a Zootherapy Intervention.
After registering the Client's needs and after inspecting the place where the activity will be held, the team draws up a protocol and shares it with the Health Manager of the Host Organization. The protocol states procedures and timetables for each intervention. Likewise, as need be, it includes health protocols and specific requests that will ensure the project will move forth harmoniously and in job security.

Working phase
The treatment protocol has to include a precise sequence of operations. On the advice of the personnel staff, a preliminary group session is held to select

patients. Patients are selected according to these criteria:

- absence of the patient's open aversion, denial, fear against the dog;
- evidence of the animal's positive behaviour response in its interaction with the patient;
- inability or refusal by the patient to perform other non-drug rehabilitation activities.

During the first leg of the project, a great deal of attention and care has to be paid to aspects such as the mutual patient-team understanding, the onset of deep and rich bonding ties and building trust-based relationships. All of these necessary and useful aspects constitute a solid starting base for any kind of therapeutic activity. It is important, therefore, to respect the pace of the relationship while accompanying the patient and the pet on their respective path to becoming familiar to one another; to foster and encourage the building of a unique and special trust-based relationship through the watchful eye of the Zootherapists who lead their pet and patients in this process of change and mutual growth as they contribute to the setting their own relational experiences with their pet, their emotions and personality style. Each relationship has its own uniqueness making it a special tool of change, growth and mutual understanding. In this stage, we even consider intervention as a tool for diagnosis and for comparison. In the initial approach to interaction with the patient, the co-therapist animal stands in as a potential detector of even the slightest emotional-behavioural alterations to the point that it can be considered as a helping "tool" useful in expanding the diagnosis (Menna, 2016a).

Remarks video-supervision
Having the duly authorizations, each intervention is video recorded. It is a valuable resource in that further observation can be conducted on the Zootherapy setting as well as the behaviour of the animal involved. Having the opportunity to review recorded sessions also implies having a second chance at analysing the animal's action and reaction to non-verbal language, through its behaviour This indeed can be a valuable resource that further contributes to diagnostic or therapeutic purposes.

3.3.1 Zootherapy Intervention: AAT according to the Federico II Model

Each AAT Intervention employs multi-strategic stimulation techniques meant to affect the patient's emotional sphere. AAT planned sessions vary according to the treatment protocol. They may include activities meant to rehabilitate areas of the mental, emotional, physical and cognitive sphere which are individually stimulated. Or, as more frequently happens, in view of their Systemic Approach, the activities involve stimulating all areas without differentiating the person from the single symptoms. It is known that, in case of rehabilitation protocols, prolonged contact with the animal (stroking or coat brushing) triggers in the patient a series of proprioceptive stimulations that travel from the periphery to the cerebral cortex through muscle fascicles, thus favouring the activation of redundancy mechanisms. Consequently, it is clear that sensory stimulation through the animal acts as a method that favours neuro-rehabilitation and thus it useful in slowing down nerve cell degeneration.

3.3.2 The structured game

Within the setting, game play with the dog contributes to creating and strengthening the therapeutic alliance. Indeed, playful behaviour is one of the most significant events ontologically and phylogenetically in the evolutionary history of animals and humans. The game, then, is to be understood as a shared space that that allows developing and strengthening a relationship based on empathy. Thanks to which, the ability to perceive and understand someone else's emotions increases and thus facilitates mirroring in someone other than itself, such as the animal. For humans the game also serves as a mediator between the drive-sensitive and the rational side so that being in front of the animal, understood as someone other than one's own self, enables the human to channel his/her emotional impulses without needing to repress them. In fact, even within the game play, compliance with rules and mutual tolerance allow to gather, without voiding, the similarities and differences between the two worlds (Rivera, 2000). The game is therefore that "potential space" (Winnicot, 1965) where the interspecific relationship gets triggered between the Veterinary Doctor Zootherapist, the co-therapist and the person.
Interventions are structured activities that are always delivered as games. The range of games is quite extensive and has its roots in Epigenetics and the in-

novative vision of Functional Neurology. Games, then, can differ in kind as long as these are designed together with the psychotherapist according to the clinical scope. All of these activities attempt to involve family and other relevant figures in an effort to create harmonious relations. The scope of these activities is to get trust, self-confidence as well as emotional opening through the game which is used as a useful metaphor in lowering defences while voiding the symptom and the disease of its medical meaning. Knowing the patient is essential even for the choice of game and its mode. Since the patient is a person, a carrier of a personal history and talents, each intervention has to take all this into account and thus be "tailored". Each Zootherapist working on the team has to know how to have the animal's and the patient's resource emerge to create the kind of therapeutic alliance which, through levity and play, achieves the designated goal (Menna, 2016a).

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Chapter 4

4.1 Population ageing and Zootherapy

The term Ageing Society refers to the current world trend in world population ageing. This trend, which is much more relevant in the so-called "mature economies", is caused by a significant increase in life expectancy, combined with a drastic reduction in birth rate. In view of this change, several disciplines, such as Neurology, Psychology, Sociology, Anthropology, and History, have developed an interest in old age. The Italian National Health System as well as its European counterpart are also resolving to take important steps. Indeed, thanks also to the multidisciplinary suggestions from these fields, they are requiring that the senile experience be re-interpreted in view of its revitalization and redevelopment. The intention to promote the resources of the elderly is beginning to take shape through a cultural shift that is starting to overcome set stereotypes. Moreover, it is also putting in place complementary health interventions meant to stimulate the old person in all dimensions (cognitive, affective, emotional and relational) so as to promote their welfare and social integration (Boudiny, 2013).

There is no doubt that if increased longevity does not proceed in parallel with good quality of life, it presents a serious problem in health spending. Of course, the fact that the elderly are physiologically subject to an inexorable and gradual decay makes their condition subject to frailty so that social care actions in their regards need to be reinforced. It is not however enough to intervene on issues, we must also promote social welfare that enables the elderly to experience their ageing in the most dignified and rewarding way possible, even through interspecific relationships and Zootherapy.

Among older people, the AAIs programs have proven to be effective in improving communication as well as in reducing loneliness and psychological symptoms linked to depression and stress (Banks&Banks, 2002; Kanamori, 2001; Kramer, 2009; Menna, 2016b). Various scientific studies have, indeed, shown the effectiveness of AAIs with regards to mood improvement, catalyzing social interactions and reducing day-to-day apathy.

Likewise, one of our objectives is to offer food for thought and a concrete demonstration as to the potential and the role of Zootherapy in line with European health and social policies whose aim is to promote the health and welfare of the elderly population

4.1.1 The scientific premises for Zootherapy in Ageing: Neurosciences, brain plasticity and lifetime learning

For a long time, it was believed that, with ageing, intellectual functions would deteriorate unrelentingly. Scientists thought that the different areas of the human brain were pre-set and immutable and that neurons ceased their reproduction after puberty. The only exception, they held, were the memory-related structures which could go on reproducing themselves well into adulthood. They drew the conclusion that once the brain reached its full development, it would become static and therefore be condemned to a slow and inexorable decline. Subsequent research has however shown quite something else. Indeed, we now know that our intellectual functions evolve over time over a an eminently individual trajectory that depends more on our life choices than on the ageing processes of brain tissue (Schaie, 1983; Shock, 1984). Our choices with regards to our living environment, the degree of education, stimulating mental activities, correct and healthy nutrition, physical exercise, good adaptive coping skills and social integration are all essential factors to maintaining our intellectual vigour. Moreover, it has been scientifically proven that through the ageing process, the human brain does lose some traits; yet these are nonetheless replaced with others that partly compensate and exceed the lost ones. The components of the peripheral and central nervous system are not irreversibly fixed in the genetic program. They adapt to environmental stress factors in every age period of the individual's life. Briefly stated, even in old age, thanks to neuronal plasticity, the brain makes up for a percentage of brain cells lost and it does so through the remaining ones which compensate for the deficit with increased branching and using alternative neuronal circuits (Levi-Montalcini, 1998). So that if increase in cognitive functioning occurs not only in puberty but throughout one's life, each individual has the ability to structurally change their thought processes and this also applies to individuals who are affected with handicaps, are heavily deprived on the psycho-social plane or if they are advanced in age. In other words brain development does not happen in terms of pre-existing instructions since its higher functions are perceptual categorization, memory and learning (Genetics); instead, connections are established and removed constantly while they undergo continuous variations that are linked with individual experience and their environment (Edelman, 2004).
Furthermore, according to Merzenich (2005), brain structure and cognitive

abilities can be improved through appropriate exercise. Brain maps are transformed on the basis of what we do in our lifetime and can change at any age, even in old age. Starting from the idea that learning consists in creating new links between neurons through their simultaneous and repeated activation, Merzenich has developed a theory that states that experience can modify the neuronal structure; this implies that even people with brain injuries or problems in specific brain areas may develop new neuronal connections.
From all that has thus far been pointed out, it is clear that what we call *competence* is nothing other than the compendium of mental activities of a lifetime; when these are put in use and exercised, even throughout old age, they activate neuroprotective mechanisms that increase the brain's longevity. The theoretical positions examined overturn the old concepts that old age is a period of stagnation and decline; instead they show that old age can indeed be experienced as a dynamic period of show that old age is not a period of stagnation and decline, but rather that it can be experienced as a dynamic period of growth.
Having taken all of these assumptions into consideration, the intent of our investigation is to verify whether specific multi-modal *interventions,* such as Zootherapy, are able to stimulate new neural connections in a health perspective that is both preventive and rehabilitative by favouring a continuation or a learning new skills even in old age. Finally, we believe that the time is ready for our proposed type of intervention. Recently, the general therapeutic field has been leaving room to a wide range of rehabilitation interventions. These we believe should be regarded as an integral part of the multidisciplinary treatment for issues related to dementia for which the time has come to draw near through a multidimensional approach (Raggi, 2007).

4.2 Evaluation of the efficacy of Animal Assisted Therapy based on the Reality Orientation Therapy (ROT) protocol in Alzheimer's disease patients: a pilot study

In 2012 sufferers of Alzheimer and other dementias were estimated to be 36 million worldwide, one million in Italy alone (Istat, 2012); these figures are going to increase dramatically in the coming years, due to the exponential rise of the elderly population. Dementia is a syndrome characterized by progressive impairment of multiple cognitive functions (phasic - gnosic - praxic), that constantly involves memory and that interferes with the performance of usual daily activities of the patient. The etiology of this disease is unknown. We know that it is a degenerative, irreversible and primary disease, caused by the progressive atrophy of the cerebral cortex, and leading to the loss of cholinergic neurons (Bergamini, 1986). Given the lack of efficacy of drug therapy based on anticholinesterase (AchEI), an increasing number of studies are investigating the validity of different techniques of cognitive stimulation to improve and slow down the progressive course of Alzheimer's disease (Viola, 2011).
As part of the rehabilitative and psychosocial interventions aimed at the person, in addition to Contextual Therapy (Colombo, 2006), Validation Therapy (Toseland, 1997) and Reminiscence Therapy (Gagnon, 1996), (Toseland, 1997), (Yamagami, 2007) one of the most common therapies used in patients with cognitive impairment is the methodology of the Reality Orientation Therapy (ROT).
The ROT is an intervention to rehabilitate of patients with mnesic deficits, episodes of confusion, and space-temporal disorientation. Its principal objective is to re-orient the patient by repeated multimodal stimulations, with respect to their personal history, environment and time. This goal can be achieved through two complementary modes of operation: formal ROT and informal ROT (or ROT in class) (Zanetti, 2004). The former consists in daily set routines when health professionals or family members interact with the patient through contact by focusing on repeated stimulation of space-temporal re-orientation. Formal ROT instead is directed at small group of patients (4-6 subjects), homogeneous in terms of cognitive impairment, for about 45 minutes a day, in a well-structured location. Currently, the ROT is one of the most widely used rehabilitation interventions for patients suffering from dementia, and at the same time, is one of the few interventions examples that have achieved positive results in patients with Alzheimer's dementia (Spector, 2001).

Frequently, patients with Alzheimer's disease are affected by a combination of disorders, mainly cognitive and mood disorders. Even if cognitive therapies are quite useful in such instances, so are therapies that are stimuli-based such as affective/motivational, emotional, and psychological stimulation(s). These conditions that facilitate the application of AAT in patients with dementia (Kawamura, 2007; Walsh, 1995). In fact, the presence of the co-therapist animal determines an affective and emotional stimulus on the demented patient; it also improves mood (Ballarini, 2003; Berry, 2012; Menna, 2012). Through the strong bond with the animal it's possible to facilitate the administration of non pharmacological therapeutic techniques in resistant patients with dementia (Motomura, 2004).
The aim of our study was to assess the possibility of using the AAT, adapted to the protocol of Formal ROT (Spector, 2001; Zanetti; 1995). For this reason, patients with mild-moderate Alzheimer disease were involved to evaluate its applicability and efficacy.

Methods

Our study was carried out at the Alzheimer Day Care Center, Asl Napoli 1 – Frullone Hospital, for six months, from January to June 2013. The head Geriatrician of the structure required an intervention of Pet Therapy because it was necessary given the difficulty of involvement of some patients in routine non-drug therapies.

The team
The team was formed by the Zootherapist (Menna, 2012), not only as responsible for the animal health and zoonoses, verifies the suitability of the setting and creates plays with the dog that reproduce the ROT stimulations. His task was to define, in agreement with the psychotherapist, the appropriate setting to the animal and to the structured play (reproducing ROT stimulations); the Psychotherapist who would establish the most suitable methods of approach to the patient and the structuring of the setting accordingly to Veterinarian. The dog, a 7-year-old female Labrador retriever, met hospital policy for participating in AAT programs (i.e. documentation of current vaccinations, controllability and temperament). The dog was trained through an educational program for the Pet therapy at the Dog Educational Center La-Voce del Cane of Naples, following the guidelines of CSEN (National Edu-

cational Sports Center, cinophilia). All procedures necessary to guarantee a high standard of animal welfare were undertaken. When the dog was found to manifest excessive calming signals (e.g. yowling, slapping) and avoiding/redirecting behaviours, the Veterinarian promptly intervened to minimize/avoid the stressing situation by changing activity, keeping distance between the dog and the stressing stimulus, or caressing the dog. This study was approved by the Ethics Committee of the University of Studies of Naples Federico II, and was in compliance with the Helsinki Declaration provisions (1996). Informed consent was obtained from all participants. Sensitive data were handled with confidentiality and securely stored.

Preliminary stage

The Head geriatrician, at the Alzheimer Day Care Center, Asl Napoli 1 – Frullone Hospital, selected at random an initial group of 50 patients (37 females and 13 males) with mild-moderate Alzheimer's disease and absence of behavioural disorders. After this first selection, the team formed three groups named AAT, ROT and Control, homogeneous for age [(mean value 75 + Standard Deviation 6 years (range 62-85)], sex, diagnosis of mild-moderate Alzheimer's disease (mean value of Mini Mental State Examination 20.1 – 95% Confidence Interval 20.0-20.1), mean value of Geriatric Depression Scale (11.5 – 95% C.I. 11.5-11.6). The AAT group were formed by 20 patients (16 females and 4 males) selected according to the following criteria: absence of rejection, fear, aversion towards the dog, acceptance by the patient of the interaction/relationship with the dog, and in addition personal history of the patient (i.e., he/she had a dog in the past). This group (AAT) received a course of Pet Therapy interventions. The ROT group formed by 20 patients (14 females and 6 males) were exclusively engaged in activities covered by the ROT. The remaining 10 patients (7 females and 3 males) formed the control group who participated in no stimulations.

Test

In order to compare the general clinical impact between the AAT group, the ROT group, and control group, Geriatrician administered the following tests to all the patients involved in this study, at time 0 (T_0) and at end of our 6-month therapies (T_1): the Mini Mental State Examination (MMSE) to assess cognitive impairment (Folstein, 1975; Mimura, 2007) and 15-items Geriatric Depression Scale (GDS) to assess depressive symptoms, and the patient's re-

actions to hospitalization and illness status (Aikman 2001; Sheikh 1986). Formal ROT. The formal ROT intervention was performed on a weekly basis, according to the protocol of the Formal ROT (Spector 2001; Zanetti, 2004; Zanetti, 1995) with sessions of 45 min, for a total period of six months. AAT adapted to the formal ROT. Each AAT intervention has been carried out once a week, for 45 min, with about the 15 initial min of presentation and approach to patient (table 4.1, Step1, AAT Intervention), 20 min of structured activity with the dog (table 4.1, Step 2 and Step 3, AAT Intervention), and the last 10 min of closing session ritualized, for a total period of six months.
The activity with the dog is structured as follows: as for the formal ROT, the therapeutic approach was based on the stimulation of cognitive functions such as attention, language skills and space-temporal orientation. The play with the animal has been structured, with some adaptations, in this way as reported in the table 4.1.

Tab. 4.1 Comparative plan of a formal ROT intervention and an AAT intervention based on the formal ROT protocol.

	FORMAL ROT	AAT Intervention
STEP 1	1. Structuring setting 2. Presentation therapist/patient 3. Stimulation of cognitive function	1. Structuring setting 2. Presentation Zootherapist/dog/patient 3. Stimulation of cognitive function trought repeated requests for information on the dog (name, breed, age, sex etc.)
STEP 2	1.Temporal orientation (Day - month - year - season) 2. Spatial orientation (Place - structure - floor - room - city - country - region) 3. Stimulation of memory	1. Temporal orientation (Day - month - year - season) refered to the session of AAT 2. Spatial orientation (Place – structure - floor - room - city - country - region) referred to the session of AAT 3. Stimulation of memory through the telling of their story about the owned dog; play structured activities with the dog: hide the ball; game of search (the ball)

continued

	FORMAL ROT	AAT Intervention
STEP 3	1. Stimulation of memory (attention) 2. Understanding of language (story)	1. Structured game with dog (attention: throwing of the ball with respect to a specific indication; hide the ball; caring for the dog) 2. Understanding of language (story: commands to the dog and waiting for the execution of the command)
STEP 4	1. Closing speech (ritualized)	1.Closing speech (ritualized: hands washing)

Tab. 4.2 Sociodemografic and clinical features of study population.

	AAT	ROT	Control	*P**
Female (%)	16 (80)	14 (70)	7 (70)	
Age (mean ± SD)	75.25 ± 6.06	74.95 ± 5.47	75.1 ± 5.83	0.987
GDS T_0 (mean ± SD)	11.5 ± 0.30	11.6 ± 0.28	11.5 ± 0.33	0.498
GDS T_1 (mean ± SD)	9.5 ± 0.46[c]	10.5 ± 0.43[b]	11.0 ± 0.46[a]	0.000
MMSE T_0 (mean ± SD)	20.2 ± 0.26	19.9 ± 0.23	20.1 ± 0.38	0.120
MMSE T_1 (mean ± SD)	21.5 ± 0.33[a]	20.2 ± 0.31[b]	20.0 ± 0.20[b]	0.000

**P*-value for differences between groups; SD, Standard Deviation; GDS, Geriatric Depression Scale; MMSE, Mini-Mental State Examination; [a, b, c] Statistycal differences between the groups by ANOVA; T_0, time 0; T_1, time 1.

Tab. 4.3 Statystical differences by Student's t-test within each group for mean value ± Standard Deviation of Geriatric Depression Scale and Mini Mental State Examination at T_0 and T_1.

Groups	GDS (mean ± SD)		*P***	MMSE (mean ± SD)	
	T_0	T_1		T_0	T_1
AAT	11.5 ± 0.30	9.5 ± 0.46	0.000	20.2 ± 0.26	21.5 ± 0.33
ROT	11.6 ± 0.28	10.5 ± 0.43	0.000	19.9 ± 0.23	20.2 ± 0.31
Control	11.5 ± 0.33	11.0 ± 0.46	0.064	20.1 ± 0.38	20.0 ± 0.20

***P*-value for differences whitin each group; SD, Standard deviation; GDS, GeriatricDepression Scale; MMSE, Mini-Mental State Examination; T_0, time 0; T_1, time 1.

Data Analisys

Data were include in an Excel file. At each time (T_0 and T_1) the mean values and SD were calculated for MMSE and GDS. Differences within groups between T_0 and T_1 for GDS and MMSE scores were analysed by using the Student's t-test. Differences of mean between groups were analysed using an analysis of variance (ANOVA) test with the Bonferroni-Dunn test for post hoc comparisons. All the statistical analyses were performed using STATA version 10.0 (Stata Corp.; Texas, USA).

Results

All the groups involved in our study were homogeneus for age, sex, and mean values of MMSE and GDS (20.1 – 95% Confidence Interval 20.0-20.1 and 11.5 – 95% C.I. 11.5 - 11.6, respectively) as showed in the table 4.2. The results of GDS, administered at T_0 and T_1, showed in the AAT group the average score decreased from 11.5 to 9.5, while in the ROT group from 11.6 to 10.5 at T_0 and T_1, respectively. In the Control group the mean the GDS score decreased from 11.5 to 11.0. Data analysis showed a statistically significant improvement in both groups, particularly in the AAT group (P<0.000) (figure 4.1). At the same time was administered the MMSE in the AAT group the mean scores were 20.2 at T_0 and 21.5 at T_1, while in the ROT group were 19.9 at T_0 and 20.2 at T_1, showing a statistically significant improvement in both groups (P<0.000) as showed in the table 3. In the Control group the mean

value of MMSE remained unchanged from 20.1 at T_0 to 20.0 at T_1 (figure 4.2). The results from ANOVA's post hoc comparisons (T_1) were reported in figure 4.3 and figure 4.4, when were showed statistical differences between AAT, ROT, and control groups. In particular, there were significant statistically differences for AAT group respect to ROT and control groups (P<0.001).

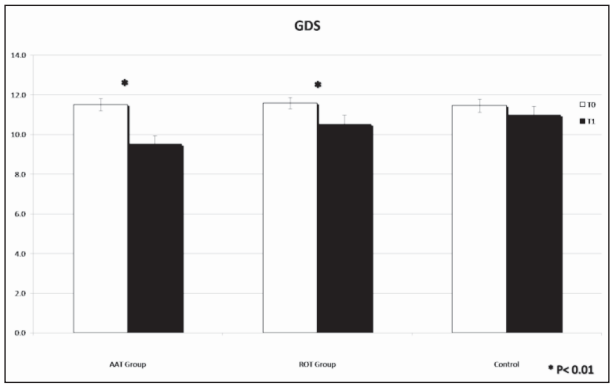

Fig. 4.1 Data analysis by Student's t-test *within each group at T_0 and T_1.*
** Significant statistically differences (*P<0.000*); GDS, Geriatric Depression Scale; T_0, time 0; T_1, time 1.*

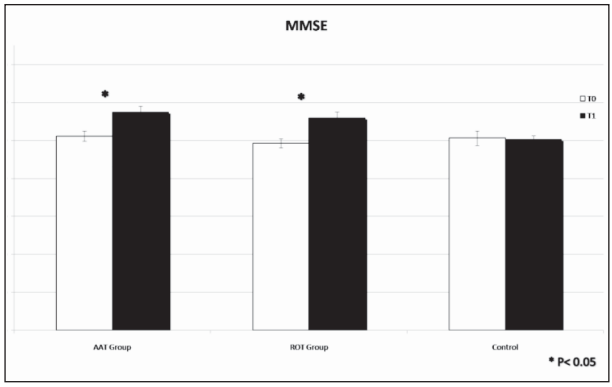

Fig. 4.2 Data analysis by Student's t-test *within each group at T_0 and T_1.*
** Significant statistically differences (*P<0.000*); MMSE, Mini-Mental State Examination; T_0, time 0; T_1, time 1.*

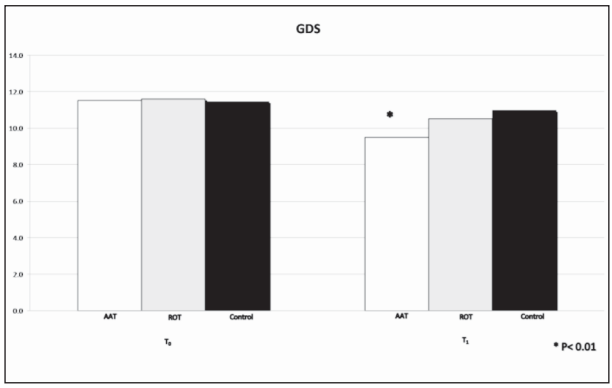

Fig. 4.3 Data analysis by Bonferroni-Dunn test *of mean differences between groups at T_0 and T_1.*
** Significant statistically differences (*P<0.001*); GDS, Geriatric Depression Scale; T_0, time 0; T_1, time 1.*

Discussion

There are several non-pharmacological therapies for elderly Alzheimer disease patients, including ROT. The average scores obtained with GDS, administered at T_0 and T_1 both to the AAT group and to the ROT group, show a slight improvement in terms of mood. In fact, considering that the test score ranges from 0 (not depressed) to 30 (maximum severity of depression), with cut-off at 11, identified by the presence of clinically relevant depressive symptoms 19, in the AAT group the average score on the GDS decreased from 11.5 to 9.5, showing a statistically significant improvement. At the same time, a slight improvement on cognitive function was observed, as measured by the MMSE, in the group AAT. Considering that the total test score ranges from 0 (very serious) and 30 (asymptomatic) with a degree of severity of the disease as mild between 21 and 26 and moderate between 10 and 20, both in the AAT and in the ROT groups showed a statistically significant improvement, particularly in the AAT group. The Control group, however, showed no significant changes compared to the MMSE and the GDS. The results of MMSE and GDS analysis showed that both the ROT group and the AAT group experienced an improvement in cognitive function and in mood, although it is clear that in the AAT group has occurred

a slightly greater improvement of the parameters, linked also to the increased presence and participation of the patient to the activities carried out with the pet. There are several non-drug therapies for patients suffering from dementia (Mimura, 2007; Walsh, 1995), but our results show that AAIs, that are structured in this way, are potentially able to improve mood and to heal the depressive symptoms of the elders involved in the study. Moreover, it has been shown that in the AAT group there was a stronger improving trend in MMSE mean scores compared with the same scores in the ROT group. The ROT is a non-pharmacological therapy accredited and widely used, can stimulate the patient mainly on the cognitive level, then starting from this premise, we have superimposed the same stimuli, adapting to structured play with the dog and going to stimulate the same channels integrating them with the stimulation on the emotional level, because the dog acts on the emotional sphere. These results show that stimulation occurred and it worked better than the ROT.

The present study also has some clear limitations. First, this is a preliminary observation in fact the study design was not randomized nor double blinded and the sample size was small. Second, we used a limited neuropsychological battery. Third, we carried out only a short-term evaluation (six months) and we cannot determine whether GDS and MMSE improvement was persistent over time. In addition, because the therapeutic effect of AAT might depend on the relationship with the animal as well as with the Zooterapist, we cannot distinguish between the differential impact of the dog and the Zooterapist on patients of AAT group. Our hypothesis is that the presence of the animal should motivate the patient to perform tasks and carry them out with improvements that were also statistically significant. In fact, the literature consulted suggest that the dog understands non-verbal language and functions as patient's emotional sounding board (Bloom, 2011; McConnell, 2006) able to produce stimulus, as demonstrated by the improvement in the results of the tests. In conclusion, AAT interventions have proven to be applicable and effective in their task to allow the continuation of cognitive stimulation and emotional improvement, intervening also in deadlock phases in formal ROT. Probably the AAT allows the non medicalization of the symptom through structured play with the dog. We hypothesize that as the ROT even the AAT through repeated stimulation multimodal (verbal, visual and tactile) in patients with Alzheimer disease mild-moderate although this work is a first observation, and requires further studies.

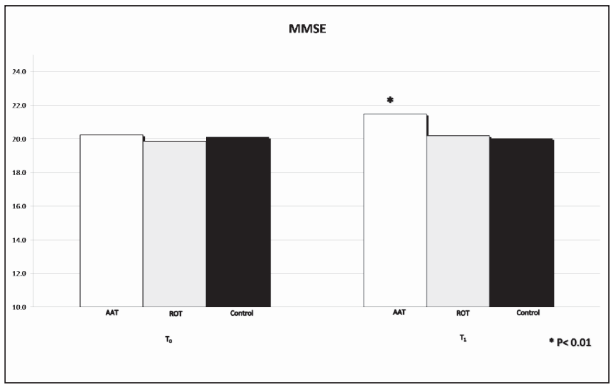

Fig. 4.4 Data analysis by Bonferroni-Dunn test *of mean differences between groups at* T_0 *and* T_1.
* *Significant statistically differences* (P<0.000*); MMSE, Mini Mental State Examination;* T_0*, time 0;* T_1*, time 1.*

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Chapter 5

5.1 Stress and Healt: the need for a holistic approach

The physiological homoeostasis of any organism is a dynamic balance kept by the interconnectedness of various biological processes and its interaction with the environment. When this balance is somehow altered, automatic responses are activated in an attempt to restore the state prior to the onset of the stressor (Monaghan, 2014). Although this is an innate adaptive response, its repeated activation response against stress can increase the risk of developing problems, over a lifetime, that threaten the survival of the organism and its quality of life. Numerous studies on research models, as each pertains to animals and humans, have shown that early adverse events are associated with a higher risk of adverse outcome in several areas of the body's functioning, negatively influencing its adaptation to the environment and therefore on his overall health status. Studies on the human research model have shown that the impact of physiological reactions to stress on the development of the individual could be an important key to understanding how negative life experiences increase the risk of developing organic diseases (Geenen, 2006; Priyadarshini, 2012) as well as mental, behavioural and affective disorders over their lifetime. Individuals, however, differ profoundly in the frequency with which they experience stressful events as well as in the vulnerability or resilience that they show in facing them (Gunnar, 2007). Some studies have highlighted that individuals react differently when subjected to the same stress. The concept of resilience, vulnerability and coping elucidate that, when introduced to a specific environment where a set of protective factors but also risk factors are present, an organism uses its own unique genetic make-up to react to events that present acute and/or chronic stress. Indeed, it should be remembered that stress is a subjective phenomenon that is based on individual perceptions, producing positive (eustress) and negative (distress) perspectives (Selye, 1936). However, an individual may switch over from feelings of eustress to mild and/or moderate distress, or even more to severe distress feelings, while indicators of mild and/or moderate distress may not be observed or may have differing degrees of severity (McVicar, 2003). Indeed, symptoms at this level of distress are likely to vary between individuals (Pastore, 2011). In a similar manner, this concept introduced in a Zootherapy context, where each of the team's members reacts individually to the various stressors that develop.

Welfare assessment involves a range of disciplines such as Ethology, Phy-

siology, Genetics and Psychoneuroimmunology (PNI) and it explores the extensive interactions involving factors (psychological and behavioural) and systems (the nervous, the immune, and the endocrine). These, in turn, provide greater understanding of the mechanisms underlying health, wellness, and diseases. Physical and psychological stressors are able to evoke immune responses that are similar to those elicited by infection from various pathogens: neutrophilia, lymphopenia, increased acute phase proteins and decreased circulating protein. Leukograms, characterized by monocytosis, lymphopenia and eosinopenia, are defined as stress leukograms. For example, Verma (1984) demonstrated that dogs subjected to acute stress develop a general leucocytosis, characterized by neutrophilia, lymphopenia and eosinopenia. There is much evidence with regards to the close relationships that involve stress, depression, inflammation, cardiovascular disease and infectious diseases. Knowing about these associations may contribute to the findings of systemic therapeutic targets and Zootherapy may offer a biopsychosocial model (Engel, 1977) to help explain the systemic interrelationships between psychosocial and bio-physiological factors (Yan, 2012).

5.1.1 Physiology of the Stress Syndrome

Stress response is a normal part of daily life, and it is only becomes harmful when triggered too intensely or for too long. Peripheral expression of the stress response is modulated via two systems, the Sympatho-Adreno-Medullary (SAM) Axis and the Hypothalamic-Pituitary-Adrenal (HPA) Axis.
The SAM Axis mediates the well-known "fight or flight" response, an initial, rapid response to an immediate stressor. Activation of the Sympathetic Nervous System and subsequent release of catecholamines (epinephrine and norephinephrine) from sympathetic nerve terminals and the adrenal medulla results in a state of physiologic readiness for response. Manifestations of the SAM Axis activation include mydriasis, increased heart rate, increased blood pressure, cutaneous vasoconstriction, an alert state, and increased plasma glucose and free fatty acid concentrations (Romero, 2007). A slower response to a stressor, whose effects can take from minutes to hours or even days, is mediated by activation of the HPA Axis leading to the release of glucocorticoids (GCs) from the adrenal cortex. This endocrine portion of the mammalian stress response originates in the Hypothalamus, with the release of corticotropin releasing hormone and arginine vasopressin. These hormones

in turn stimulate the release of adrenocorticotropic hormone from the pituitary gland, resulting in the production and release of GCs from the adrenal glands. Peripherally circulating GCs, cortisol and corticosterone, provide negative feedback to this system (Romero, 2007). Glucocorticoids influence a large number of metabolic processes (including protein, glucose, and fatty acid metabolism, and immune function), and can induce a catabolic state, while corticotropin releasing hormone suppreses gastro-intestinal motility and arginine vasopressin regulates the glomerular filtration rate (GFR), cAMP generation, and fluid balance. Acting jointly, these hormones can also influence growth, thyroid function, and reproduction (Hekman, 2014).
Hans Selye, a Hungarian physician, was one of the first scholars to focus attention on mechanisms that the body activates when it undergoes a stressful event. He developed a theory whereby the organism activates a *"General Adaptation Syndrome"* (Selye, 1973). Of it, we single out three phases:

1) the alarm phase, or rather, the first reaction the body has upon perceiving the stressful event (heart rate and breathing increase/vasodilation and vasoconstriction phenomena).

2) the resistance phase, during which the body attempts to adapt to new conditions to restore its equilibrium. This phase is characterized by the activity of both the Hypothalamic-Pituitary-Adrenal Axis and, especially, of the glucocorticoids which are mainly responsible for the organic reactions and behavioural problems that occur.

3) the collapse phase, when the persistent stressful conditions result in the individual's inability to react. In this third and last phase, the body has reached such a debilitation degree that infectious and/or organic diseases easily attack it. Likewise, the situation can become so serious to even lead to a fatal outcome.

Each of these phases is characterized by neural, hormonal and humoral modifications which explain the behavioural and clinical consequences that we observe (Sechi, 2011; Selye, 1973; McEwen, 1998).
The organism, we could say, has the ability to put in place a series of organic and/or behavioural responses whose goal is restoring its normal homoeostasis against "stressors" which can be defined "any factor causing changes in the

normal homoeostasis". Should this reaction not occur, the subject's natural equilibrium would fail and thus let the typical acute stress symptoms to set in; in the event of a persistent discomfort, chronic stress symptoms would follow which could lead to a more or less severe pathological condition (Sechi, 2011).

5.1.2 Biochemical stress measurement: a predominant role of cortisol

Psychological stressors are the most potent natural stimuli known to affect the Hypothalamic–Pituitary–Adrenal Axis (HPA; Mason, 2004). Some specific stressors that influence the HPA include exposure to new or threatening surroundings (Friedman & Ader, 1967), separation from attachment objects (Hennessy, 1997), unpredictability of external events (Muir&Pfister, 1986), and loss of control over the environment (Hanson, 1976). Cortisol is considered one of the major indicators of response to stress in most mammals, including dogs (Kirschbaum & Hellhammer, 1989), and is used in animal welfare studies to detect poor welfare (Beerda, 1996; Pastore, 2011). In mammals, cortisol plays a major role in the response to altered internal or external stimuli. Regarding its primary function, cortisol requires modulation of bodily functions to maintain homeostasis under novel conditions. However, prolonged exposure and/or excessive secretion of cortisol may lead to clinical symptoms and stress-adaptive disorders (Glenk, 2013).
Scientific literature on stress has employed several biochemical markers to evaluate the functioning of the Hypothalamic–Pituitary–Adrenal (HPA) Axis. In recent years, cortisol has been "elected" as the main indicator in this regard. One of the main roles that cortisol plays on the organism is its action on the immune system, especially noticeable during Selye's Collapse Phase as well as during chronic stress. A recent study showed that, depending on the type of stressors present, the stress management triggering varies in the brain stem, especially in regulating cortisol secretion (Dedovic, 2009). For example, a response stressor, which can be represented by a real sensory input (such as the subject's pain, physical damage or immune problems), tends to trigger the Medulla Oblongata, and more particularly specific Hypothalamic nuclei. Meanwhile the anticipatory stressors, meaning those triggered by innate or by memory-related reactions (such as social conflicts or unknown situations) mainly trigger regions of the limbic system, such as the Hippocampus, the Amygdala and the Medial Pre-Frontal Cortex (Herman, 2003).

Cortisol is extensively used in measuring Hypothalamic-Pituitary-Adrenal Axis (HPA) activity and is found in plasma, saliva, feces, urine, and hair of many species.

Salivary cortisol is considered a biomarker of psychological stress that evaluates both the functioning of the HPA Axis in response to stress and the basal circadian rhythm of the cortisol secretion (Hellhammer, 2009).
Salivary cortisol concentration has a good correlation with the concentration of the unbound fraction of blood cortisol. Saliva in fact is a natural ultra-filtrate of blood, and plasma cortisol not bound to proteins transport freely flows into saliva by passive diffusion through the glandular epithelium. This is done independently by the molecular kinetics of any transport mechanisms, and by the salivary flow, thanks to the low molecular weight (PM 362) and to the poor polarities that characterize the cortisol molecule. The absolute values of salivary cortisol are minor compared to those of the unbound fraction of blood cortisol subsequent to the action of 11βHSD2, abundantly expressed at the level of the salivary glands. In general, we can consider that the concentration of cortisol in saliva reflects very well the serum fraction of free and biologically active cortisol (Gröschl, 2003; Hellhammer, 2009).
Since the quantities of salivary cortisol are very low, the methods to apply in this measurement shall be suitably sensitive and precise. The peculiarity and the great advantage of measuring cortisol via saliva rest in its simplicity, in its non-invasive nature, in the repeatability at short intervals and, last but not least, in its low cost. Sample collection is, in fact, non-invasive, non-risky and stress-free, and the patient can do it himself in a relaxed environment. The non-invasive specimen collection makes the technique suitable even for children and the elderly, especially in cases of phobia of needles and guarantees lower ethical problems in experimental studies (Törnhage, 2009).

5.1.3 Salivary cortisol measurement in dogs

The relationship between stress, health, and well-being of domestic dogs is a significant area of scientific interest concerning canines in both companion and working contexts (Cobb, 2016). Salivary cortisol has been increasingly used as a measure of stress response in dogs and has found particular favor in canine studies on welfare and human–animal interactions (Bergamasco, 2010; Dreschel & Granger, 2009; King,2011).

Salivary cortisol collection in dogs does not alter the activity of the HPA system itself but it is a potent marker in detecting physiological responses to a stressful stimulus (Dreschel & Granger 2005). Saliva collection is relatively non invasive and can be collected and stored at convenient and meaningful times of the day in a variety of settings. Although a number of studies have failed to show a diurnal rhythm in plasma or salivary cortisol concentration in dogs, Beerda et al (1999) recorded canine subjects as having significantly higher mean of salivary cortisol concentrations in the morning than throughout the rest of the day.

Although animal handling is required, saliva collection is tolerated well by most dogs and is not technically challenging, allowing people to be easily trained to collect samples. Collection involves saturating absorbent collection material with saliva in the dog's mouth, with or without the presence of a salivary stimulant. It has been shown that if the collection procedure takes <4 min, there is no handling effect on the cortisol concentration of that sample (Kobelt, 2003). Saliva is then extracted from the absorbent material, frozen for an interim period, and tested for cortisol concentration using validated radio- or enzyme-linked immunoassays (Cobb, 2016).

With the increasing use of salivary cortisol concentration as a measure of canine stress and welfare, it is important that literature sets range limits [salivary concentration range of 0 to 33.79 μg/dl, median (0.15 μg/dl), and mean (0.45 μg/dl) values] and identify any canine, environmental and experimental effects on this popular marker (Cobb, 2016).

5.2 Dog behaviour in case of stress

Behavior can be held as the physical manifestation of an animal's current physical and mental health state (Broom, 1991). Likewise, abnormal behavior can be an indicator of poor animal welfare, as different behaviors range from very good to unacceptably poor welfare. A threat within any given context, condition, interaction or event can give way to stress and to negative responses in animals, which manifest it as changes in behavior (Mason, 2004) and all this, can happen also in AAI setting.

The behavioral observation is a good diagnostic measure since its non-invasive nature allows to further delves into the animal's wants, needs, and internal processes without disturbing its natural state (Mason, 1991). At this point, we must focus on behaviours in dogs undergoing acute and/or chronic stress considering the ethogram of animal species which consists in a catalogue of behavioural repertoire (or behavioural module) of the animal.

Behavioral stress indicators range from normal to abnormal behavior patterns. Dogs' normal behavioural responses to acute stressors are: avoidance, defensive aggression, hiding, seeking contact with humans or other species, attention-seeking behaviours (i.e. pawing), excessive activity or inactivity, digging, panting, salivation, elimination, pacing, visual scanning, dilated pupils, vocalization, lowered posture, flattened ears, low tail, anorexia (Casey, 2002).

Moreover, there are some fear-related behaviours which include: ears ears brought back (sometimes even completely flattened on its head), lips pulled back (without showing teeth), eyes ajar (narrow, winking), limb flexion (body crouches), tail down (sometimes completely tucked in between the hind legs) (Abrantes, 2008).

Environmental stress, such as forced isolation and in cramped spaces, usually causes dog to perform repetitive movements and actions such as walking without a purpose, chasing its tail. Beerda in one of his studies states that an increase in some behavioural modules such as body shaking, crouching, using the mouth, yawning, agitation and low posture are behavioural indicators of acute stress. The intensity and type of behavioural response depend very much from the stressors that caused it. Stimuli that cause predictable stress (such as opening an umbrella) or that need human presence in order to occur induce various responses such as agitation, moderately low posture, frequent body shaking and "oral behaviour" (Beerda, 1998). In this specific regard, it

has to be emphasized then that many of the dog's behavioural modules have specific communicative meanings that could be associated with "calming signals". Of those known, there are around 30 that or so signals that, generally speaking, dogs use to convey a peaceful emotional state to the intended recipient of the message. It frequently happens, however, that dogs use them either to prevent or stop something or to calm themselves down when they feel stressed or uncomfortable. Some calming signals are: head spinning, looking away to avoid a direct eye contact, squinting, licking the snout, yawning, sniffing (Rugaas, 2006).
In conclusion, a state of acute stress may indeed be identified through a behavioural assessment. Yet, in addition to considering the type of stressors and the dog's individual characteristics, the simultaneous evaluation of physiological parameters can definitely help avoid misunderstandings (Beerda, 1998). For example, the dog is particularly sensitive to certain stressors (such as sudden bursts, bag falling) which cause it to assume a very low posture, associated with high blood levels of cortisol (Rothuizen, 1993).
Instead, in as far as dogs' behavioural modules shown after chronic stress, applying studies appears to be more controversial, especially for ethical reasons. In terms of chronic stress and compared to dogs experiencing less austere conditions, dogs subjected to social and spatial restriction for extended periods displayed abnormal behaviours such as enhanced locomotion, yawning, paw-lifting and body shaking (Beerda, 2000) along with increased self-grooming, coprophagy, low posture and vocalizing (Beerda, 1999).

5.2.1 Stress and coping

The term "stress" covers different concepts: physiologic stress, non-physiologic or psychogenic stress, and distress (Ward, 2008). Physiologic stress describes exposure to positive or negative physical, systemic, or environmental challenges that perturb the body's homeostasis. In a veterinary setting, negative physiologic stress may be induced by systemic illness, trauma, and surgery. Similarly, psychogenic stress describes exposure to psychological or social challenges which result in psychological well-being being disrupted. Negative psychogenic stress in a domesticated animal may be induced by several factors: separation from a caretakers; being subjected to invasive procedures in the absence of familiar caretakers: or being exposed to a new environment. Positive psychogenic stress has been less widely studied but

may be understood as instances such as being reunited with a caretaker or becoming engaged in a highly anticipated game such as fetch. Both physiologic and psychogenic stress are a normal part of life, and a healthy body and mind can adapt to keep normal function (Sapolsky, 2000). Such a state becomes distress when the burden of physiologic stress is too high and the body is unable to restore its homeostasis, or when overwhelming psychogenic stress threatens the person's mental well-being. When marked, stress is associated with numerous pathophysiological sequellae, ranging from poor mental to poor physical well-being.

Lazarus and Folkman (1984), in their Cognitive-Transactional Model on "stress" and "coping", focused on the subjective perception of the stressful event and the individual's ability to cope with it. According to their theory (Berjot & Gillet, 2011; Lupis, 2014), stress is conceptualized as a dynamic element and given its relational traits, it becomes blended into "psychological stress". Despite this, however, stress is not an exclusively subjective experience, inasmuch as its extent is also defined by the objective traits of the stimulus. The stressor intensity of an event is therefore determined not only by the cognitive assessment of one's own stimulus but also by the objective characteristics of the stimulus (Goldstein, 2007). Finally, the stressor intensity of an event is defined not only by the cognitive assessment and the emotional perception of the stimulus (Primary Appraisal) but also by the individual's assessment of resources and ability to cope with stressful stimulus (Secondary Appraisal).

Known as "coping" strategies, these modalities refer to the adaptation process in a stressful situation, even though they do not guarantee that the adaptation will be successful. More recently, several author delved into the various coping strategies that individuals can put in place when faced with stressful situations. According to them, the different coping strategies that are part of an individual's background interact amongst each other so as to obtain positive results. In other words, successfully adapting to stressors will depend on how the different coping styles interact with each other and the situation before them. This leads to the consideration that, in reality, there are no adaptive or maladaptive coping styles *a priori*. This much is said because although some strategies may be effective in one situation, they may not be so in another; just as, reactive modes that are otherwise positive if used moderately and temporarily, can actually turn into negative modes if used exclusively (Zeidner & Saklofske, 1996). Psychological research has yet to settle on this

topic. Nonetheless, to date, it emphasises that a good adaptation to stress, especially in the case of long-lasting stressful events, requires flexibility in the use of coping strategies. Flexibility is then the essential element for coping, or rather not to get hung up on a single strategy but to find alternatives to it and change course if it proves ineffective and/or maladaptive (Sgaravatti, 2014). The flexibility of Zootherapist/dog and patient is also the essential condition for the management of a Zootherapy setting.

5.3 Zootherapy as an wellness-promoting intervention in patients affected with Dementia and depression via salivary cortisol measurement in the Veterinary Zootherapist, co-therapist dogs and in the patients: a preliminary study

The practice of involving dogs in treatment settings is on the increasing as quite a lot of literature suggests in its attempt to link the human-animal relationship during AAIs with important parameters for physical and psychological health (Friedmann, 2012). Although AAT is by definition a significant part of the therapeutic process (Kruger & Serpell, 2006), the protection of the animal well-being in AAIs so far has received very little attention (Hatch, 2007; Marinelli, 2007). Implications for the well-being of dogs can derive from the interaction with strangers in unfamiliar settings, forced positions with no possibility to escape, and/or inadequate methods of formation (Hatch, 2007; Piva, 2008). As for the AAIs, accurate studies are needed to evaluate the effects of human-animal interactions in the co-therapist dogs (King, 2011). Preliminary investigations (Marinelli, 2009;. King, 2011) and anecdotal reports of case studies (Piva, 2008) have presented a conflicting framework with regards to potential implications on the dog well-being in therapeutic settings.
In order to promote a systemic view over the well being of the single components in a setting, the aim of this preliminary study was to assess the Zootherapy benefits by monitoring the levels of salivary cortisol not only as a stress indicator but also as a well-being parameter in elderly patients affected with mild Alzheimer's, in Veterinary Zootherapists and co-therapists dogs involved in the settings.

Materials and methods

Preliminary phase
In this first phase, the Medical Director of the hosting Unit selected patients that were considered suitable on the basis of the clinical and behavioural parameters that the project required. At the same time, the Zootherapy team, consisting of the Veterinary Zootherapist with his/her co-therapist dog and the Psychologist expert of interspecific relationship (IRE), made a further selection of patients.
It used the criteria that follows: absence of denial, fear, aversion; acceptance

of interspecific interaction/relationship; personal story. Moreover, in according to the objectives set, the Zootherapy team developed a specific Protocol to define frequency and timing for the entire intervention Cycle.
Lastly, the team selected the most suitable space where interventions were to be heald just as it structured the setting according to the needs of the co-therapist dog and the Zootherapy team (Barker, 1968; Menna, 2016a).

Study design
Our study was carried out at the Day Center of Villa Walpole's Geriatric Ward (ASL-NA 1) from March to June 2016.
The Zootherapy Cycle lasted 3 about months and its weekly interventions were about 20-minutes.
The team included 6 Zootherapists and 5 co-therapist dogs.
Twelve adult patients were selected: (7 females and 5 males) homogeneous for age [(mean value 75 + Standard Deviation 6 years (range 62 - 85)], diagnosis of mild-moderate Alzheimer's disease (mean value of Mini Mental State Examination 20.1 – 95% Confidence Interval 20.0-20.1) mean value of Geriatric Depression Scale (11.5 – 95% C.I. 11.5-11.6). The 12 patients who met the inclusion criteria were randomly assigned to three groups (A – B – C) each comprising 4 individuals. **Group A** carried out Zootherapy activities in individual setting. **Group B** played an indirect and unstructured activity with the animal present. **Group C** did not take part in any activity involving animals (control group).
Each Zootherapy intervention was videotaped so as to monitor the behaviour of the dogs involved.

Study animals
The dogs selected had gone through an educational training program that adheres to the Guidelines set the Italian National Educational Sports Centre (CSEN). The co-therapist dogs selected were 5, of which: 1 females (spayed) and 4 males (1 neutered). Breeds included Dachshund (n=1), Cavalier King Charles Spaniel (n=1), mixed breed (n=3). The 5 dogs ranged in age from 2 to 6 years with a mean (± SD) age of 3 (± 4.1). Therapy protocols stipulate that all therapy dogs have to undergo regular veterinarian screening. The dogs were considered healthy based on the medical history and results of physical examination (ie free from pain, endo- and ectoparasites and immunised).

Operational phase

The Zootherapy interventions were multi-strategic in nature and were made up by structured activities that the Zootherapist-co-therapist dog administered as game plays (*structured play* for A group; *unstructured play* for B group) in relation to the objective sought. The activities were organized as part of the emotional/affective, motivational and motor activity dimension of the person, in an individual or group setting.

Sample collection

Saliva samples were taken from patients, Zootherapists and dogs. To absorb the saliva, we used Salivettes® (Sarstedt, Rommelsdorft Corp., Germany).

The samples were collected using a commercial saliva collection kit (Salivettes®, Sarstedt), with the Salivette remaining in the person's mouth for 1 min per sample. Care was taken when collecting saliva to avoid collection immediately after mouth cleaning, meals, snacks, or medications (Hodgson, 2004). The saliva collection device was gently placed into the cheek pouch or under the tongue of the dog until it was saturated with saliva (approximately 1 min). For ethical reasons, none of the dogs were restrained during the sampling procedure, indeed salivary samples were collected within 4 min (Kobelt, 2003). To stimulate salivation, each Zootherapist presented commercial food to their dogs. In order to avoid sample contamination and, hence, reduced reliability of the enzyme immunoassay, the dogs were only allowed to sniff at the food treats in the experimenter's closed hand and not to chew on it (Bennet & Hayssen 2010; Ligout, 2010).

The collected material was stored in an ice box prior to samples being taken in the laboratory within an hour's time from its being taken. Likewise, prior to analysis, these were thawed and centrifuged at room temperature at 3,000 g for 5 minutes to obtain the clear saliva sample in the conical tube. Subsequently, the cortisol salivary levels were measured by chemiluminesence technology (IDS-iSYS Salivary Cortisol) according to the protocol for salivary samples.

Sampling schedule
Home baseline salivary sampling was carried out on a day without Zootherapy sessions at 6.30h. Saliva samples were taken from patients (n=22), Zootherapists (n=6) and dogs (n=5) (Beerda, 1999).
To lessen the effect of potential circadian deviation on salivary cortisol, only intervention starting in the morning from 09.30-11.00h were considered in the analysis.
This study was approved by the Ethics Committee of the University of Studies of Naples Federico II, and was in compliance with the Helsinki Declaration Provisions (1996). Informed consent was obtained from all participants. Sensitive data were handled with confidentiality and securely stored. Upon the Unit Management's approval and upon patients' informed consent, each Zootherapy meeting was filmed. Each video clip received supervision by the team.

Statistical analysis
Data were include in an Excel file. At each time (T_0 and T_1) the mean values and SD of salivary cortisol levels were calculated. Differences for Zootherapist Veterinary, co-therapist dog and patients between T_0 and T_1 for salivary cortisol levels were analysed using the Student's t-test for paired data. In order to analyse the relation existing between the quantitative variables amongst the single groups (Zootherapist/dog, dog/patient, Zootherapist/patient), we used the Pearson correlation coefficient. Statistical analysis was performed using SPSS 13 software for Windows (SPSS Inc., Chicago, IL, USA).

Preliminary results
Preliminary results of behaviours observed by the dog in the Zootherapy setting

We conducted additional observations of the three different Zootherapy groups (Group A-B-C) via the Solomon Coder (https://solomoncoder.com/), a program for coding behaviour of the dogs by assessing the ethogram (Abrantes, 1997).

From an initial observational analysis we are able to state that the behavioural modules observed can be classified as:

- **stereotypy** (Broom, 1998): we are referring to behaviours that can be car-

ried out through different behavioural modules; for example, dogs can turn in circles or chase their tail (Moberg, 2000).

- **displacement activities** intended as behaviours put in place without a having functional relevance. For example, the response may be licking a paw or a side (Troisi, 2002).They can be generated by conflict situations when the animal "wants to do something, but it isn't allowed to." Environmental stimuli that the animal perceives as unpleasant or dangerous may result in an internal conflict whose outcome may be an "out of context" behaviour.

- **apathy**: dogs show little or no exploratory behaviour; they are unresponsive to social stimuli; or, they can even be indifferent to an extremely adverse situation (Broom & Johnson, 2000).

A threat within any given context, condition, interaction or event can give way to stress and to negative responses in animals, which manifest it as changes in behavior (Mason, 2004). In an AAI setting, for example, a dog's behavioral response to human contact can be highly variable. Indeed, our observations on the dog showed an adaptive and behavioural flexibility of each animal (coping). Indeed, although behavioral response strongly depends on the individual dog, a human's appearance and demeanor also influences how a dog will respond. Moreover, the age and sex of human has been found to elicit different responses in dogs. As a matter of fact, we observed that the older the dog was and the more experience it already had with settings, the lesser signs of stress it showed along with its displaying no apathy. Having said this, we must also add that such a display might be due to a "habit" process towards the therapeutic setting (King, 2011). Furthermore, our preliminary observations showed that along with stress signals, there were frequent interactive behaviors (dynamism, wagging); nonetheless, these might be due to the fact that stress-related behavior in dogs was shown as more evident when interacting with children under 12 than with elderly clients (Marinelli, 2009).

Results

A total of 460 salivary samples were collected from Veterinary Zootherapists (n=100), 5 healthy dogs (n=120), patients (n=240). 28.26% samples yielded

an insufficient amount of saliva and could not be used for analysis.Reasons for these failures included failure to chew the cotton pad. Moreover, occasionaly, elderly did not agree to sampling being taken.
Instead, in dogs, reasons for these failures included defense movements, such as head shaking, backing off, and pawing at the clamp, failure to chew the cotton pad, or excessive chewing resulting in destruction of the cotton pad.
The greater part of the singleinterventions in each group (A-B-C) showed a decrease in salivary cortisol between T_0 and T_1. Yet, the average salivary cortisol mean for each group at T_0 and T_1 in each of the 10 meetings (table 5.1), showed no statistically significant results except in the co-therapist dogs of group A (P <0.05).

Tab. 5.1 Statystical differences by t-test of paired data for mean value ± Standard Deviation of salivary cortisol levels at T_0 and T_1.

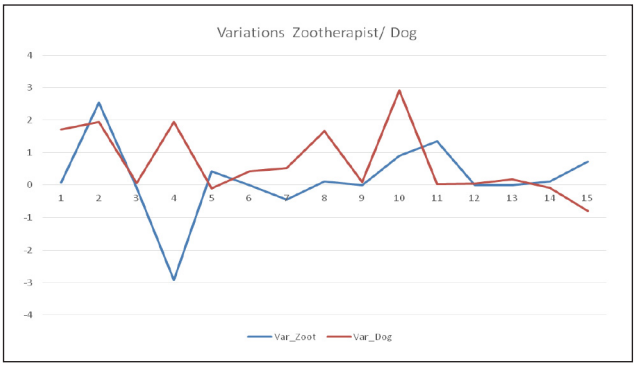
Groups	Zootherapist (mean ± SD)		*P***	Co-therapist Dog (mean ± SD)		*P***	*Patients (mean ± SD)*		*P***
	T_0	T_1		T_0	T_1		T_0	T_1	
A	1.51 ± **0.84**	1.14 ± **0.57**	**0.169**	2.56 ± **0.31**	1.57 ± **0.37**	**0.013**	0.65 ± **0.37**	0.80 ± **0.61**	**0.421**
B	1.34 ± **1.02**	1.20 ± **0.76**	**0.548**	1.39 ± 0.53	0.77 ± **0.65**	**0.453**	0.47 ± **0.45**	0.69 ± **0.53**	**0.525**
C	-	-	-	-	-	-	0.87 ± **0.52**	1.13 ± **1.08**	**0.323**

**P-value for differences of salivary cortisol levels (μg/dl) at T_0 and T_1; SD, Standard deviation; T_0, time 0; T_1, time 1.

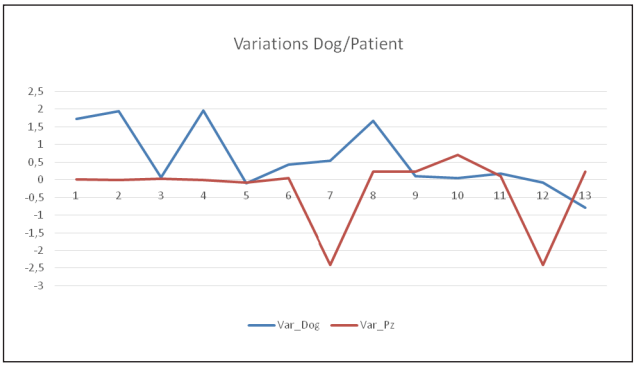
Discussion

The study was designed to investigate short-term effects of human-animal interaction on salivary cortisol levels in Veterinary Zootherapists, dogs co-therapist and patients. In each group (A-B-C), saliva samples were collected before and after therapy session.
The variations in salivary cortisol within each group and for each partecipant (Zootherapist Veterinary, dog co-therapist and patient) did not give significant statistical results in the different sampling times (T_0/T_1). It is probable that this may have occured due to the quantities of salivary cortisol being very low and considering that the sampling was small.
At the same time, however, we believe that our data are both interesting and worthy of further study. Despite the lack of significative statistical data, the slight reduction of salivary cortisol observed as this pertains to each intervention session and each group does reinforce some of the theses found in our research. We know, in fact, that the positive interaction with humans, quiet play and affiliate behaviours were linked to reduced cortisol levels in dogs (Glenk, 2013; Horváth, 2008). Moreover, considering that in dogs, cortisol rise has been associated with stressful conditions resulting from fear, controlled/authoritarian play and human threat (Glenk, 2013), we can hypothesize that the educational of our Zootherapist and the training of our dogs worked to prevent a stress condition within each group from becoming stable or worsening. Lastly, it should be remembered that stress is a subjective phenomenon that is based on individual perceptions (Selye, 1973). However, an individual may switch over from feelings of eustress to mild and/or moderate distress, or even more to severe distress feelings, while indicators of mild and/or moderate distress may not be observed or may have differing degrees of severity (McVicar, 2003) and this would confirm the slight and yet constant reduction of salivary cortisol in Setting A and B with regards to both the Zootherapist and for the dogs and patients. The same trend, furthermore, did not occur for patients in Group C which was serving as the control group.
Moreover, in evaluating the differences between the salivary cortisol values between T_0 and T_1 for each Zootherapist, dog and patient in Group A, through the Pearson coefficient, we noted a scarce correlation, or rather the cortisol variations did not follow the same pattern among the three subjects (graphic 5.1, 5.2 and 5.3, respectively).
This piece of data suggests to us to delve further in investigating the above

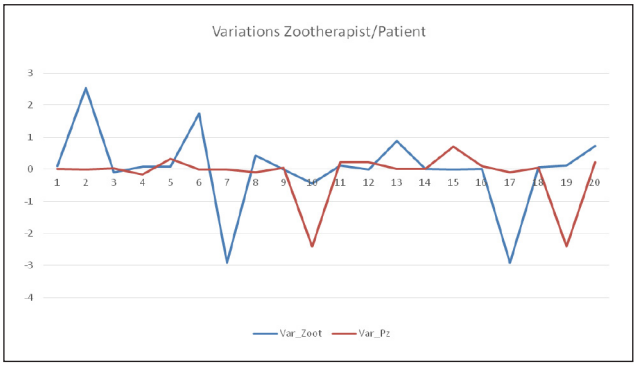
mentioned evaluation about differences of salivary cortisol values both by widening the number of subjects involved Zootherapists, co-therapist dogs and patients) and by extending the treatment period so as to single out any potential periods/critical phases in relation to the time factor and on the basis of the quality/quantity of the interspecific relationship administered throughout the entire Zootherapy cycle.



Graphic 5.1 Variations Zootherapist/dog
Correlation coefficient: -0.04



Graphic 5.2 Variations dog/patient
Correlation coefficient = -0.113



Graphic 5.3 Variations Zootherapist/Patient
Correlation coefficient = 0.074

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Chapter 6

6.1 Zootherapy and prevention

Although "successful aging" was not an explicit theme in the biomedical literature until the early 1960s, there have long been efforts to understand how to promote longevity and positive states of health in later life. Aging has been described as a largely intractable process versus one involving possibilities for adaptation to new roles. Modern psychiatrists and psychologists considered later life either as a product of early developmental tasks or as a period of continued growth and conflicts that had to be negotiated (Jeste, 2010). In their influential 1987 article, Rowe and Kahn noted that research on aging was historically dominated by efforts to discriminate between pathological and "normal" aging, with little effort being devoted to understanding the upper end of the continuum (i.e., successful aging). Definitions of successful aging are all multi-dimensional and integrate multiple domains: a) freedom from disease and disability, b) high cognitive and physical functioning, and c) emotional and social functioning (Jeste, 2010).

The International Health Systems are moving while having in mind an integrated and multidimensional approach towards the elderly through prevention interventions at primary, secondary and tertiary levels (Auxilia & Pontello, 2012). The approach to health prevention for older people, then, can only be multidimensional; an approach, in other words, that foresees a kind of global bio-psycho-social intervention which, in a systemic perspective, adopts a multidisciplinary approach to health promotion that integrates the patient's biological, psychological and social aspects (Engel, 1980).Yet, whereas the broader aim is to minimize the main risk factors and promote appropriate lifestyles in all age groups, these interventions are to specifically focus of maintaining self-sufficiency and quality of life in the elderly.

Through the animal, Zootherapy supplies affective/motivational and cognitive stimulation, emotional, motor skills. In addition to having a mediator role, the animal is a social and emotional motivator so as to create renewed desire for communication and socialization in the elderly (Barker, 2010). In addition, several studies have shown that the co-therapist animal, and specifically the dog, has a role of "emotional mediator", " facilitator and social catalyst that is able to act both on the affective and emotional sphere of the older person (Wilson & Netting, 1983), thus encouraging an increase in verbal interactions and socialization (Fick, 1993) as well as an improvement to mood disorders (Menna, 2012). Indeed, the warm empathetic emotions and

interspecific may be effective in consolidating or recovering cognitive, motor and expressive potentials which are of support to a person's well-being, in accordance with the concept of One Health designated by the World Health Organization.

Based on this we hypothesize that, when integrated in a prevention programme, Zootherapy may be able to implement the health of individuals and community at large through prevention programs (*primary prevention*) in order to reduce individual risk factors related to ageing (Auxilia & Pontello, 2012).

6.2 A project in health prevention and well-being promotion in older healty individuals by enhancing the symbolic value of the dog and through the measurement of salivary cortisol in the Veterinary Zootherapist and in the elderly: a preliminary study

Ageing does not necessarily imply a decline in emotional capacity, better yet the elderly sharpen their emotional intelligence as years go by (Tsai, 2000). In the most recent years, emotional competence has been receiving growing interest due to its implications in correlation to diverse evolutionary paths. Emotions have evolved in a cross-species manner to favour achieving those motivational dimensions related to the individual's interests and welfare (Ekman, 1992). Emotions, it could be said, are important adaptation tools that an individual has to get connected with his natural and social environment. These tools allow achieving a greater state of well-being while improving one's own emotional skills which, especially in the elderly, are at major risk because of tendencies to isolation and depression. At first sight, the trajectory of emotional aging may appear surprising. Given that older adults are confronted with bodily deterioration, increasingly frequent health problems and memory failures, and losses in mobility and in the social worlds, how do people maintain high levels of affective well-being? One possible explanation, which has received much attention, is an increasing motivation to regulate emotional states and increasing competence to do so (Scheibe & Carstensen, 2009).

Our project consisted in a series of meetings whose aim, in an interdisciplinary perspective, was to promote a greater awareness of the resources and skills they already held and to improve their empathic abilities. This goal was achieved through a structured course during which new ways of learning and of expressing were explored by enhancing the symbolic meaning of both animal and interspecific relationship.

We know that the dog, in the relationship entertained with a person, intervenes as it stimulates processes of development and change that act at pre-symbolic level through its mediating role of the symbolic experience (Sangalli AL, 2005). During our meetings, in fact, the animal's figure was only evoked and described by Zootherapist so as to promote a kind of "emotional exercise" that can be applied to everyday life through the discovery of new repertoires that are relational and emotional alike.

Materials and methods

Study design

Our study was carried out at the Santa Maria del Buon Consiglio Parish Church, Confalone di Napoli, from March to June 2016.

The project lasted about 3 months and its weekly interventions were about 45-minutes.

The project consisted in 10 meetings conducted by a Veterinary Zootherapist for a group of 10 healthy elderly volunteer subjects (7 females and 3 males) who had a mean age of 65 years (range, 55–68 years). Inclusion criteria were willingness to interact with dogs, indeed the most subjects were current pet owners (n = 6) and/or had owned pets in the past (n = 4). The main exclusion criterion was fear of dogs.

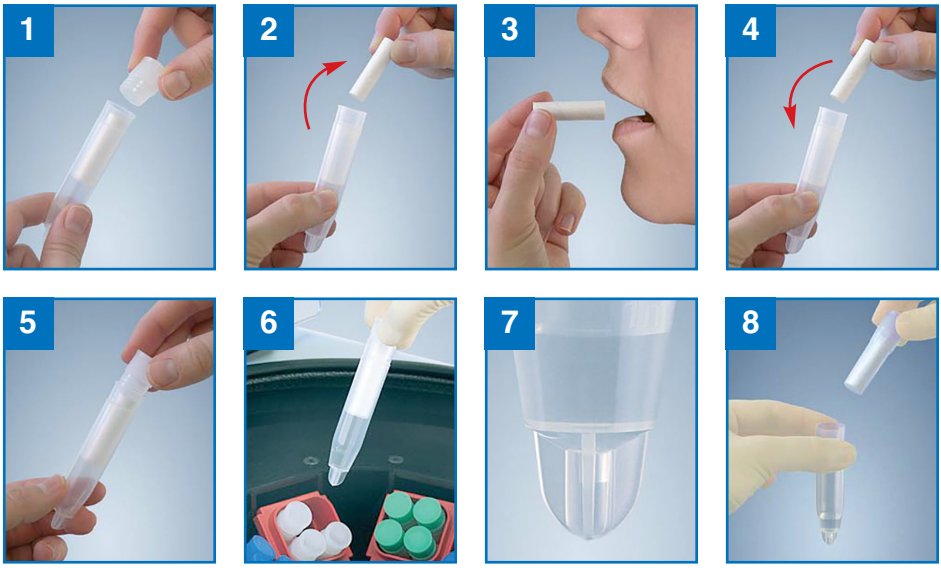
Operational phase

Our meetings focused on activities that were *reference* type during which the elderly were directed to develop and interpret a new relational dimension without the actual physical presence of the animal. During the meetings, animals were evoked symbolically through a teaching approach about anatomy, physiology and behavioural model; with basic information on dog training, prophylaxis and getting tested for zoonoses and through an emotional approach mediated by images, pictures, roles plays, drama and non-verbal communication exercises. Each meeting session ended with a ritual which consisted in recounting an Zooanthropology tale. In such a participant served as the narrator and the rest of the group served as actors in the play.

Sample collection

Participants were told not to eat, drink beverages containing caffeine, sugar or fruit juices, smoke, brush their teeth, or use mouthwash in the 30 minutes prior to salivary collection (Mantella, 2008). All subjects also received written instructions. The samples were collected using a commercial saliva collection kit (Salivettes®, Sarstedt), with the Salivette remaining in the person's mouth for 1 minutes per sample. Two samples were collected from Zootherapist and from each elderly before and after when the meeting had finished. The collected material was stored in an ice box prior to samples being taken in the laboratory within an hour's time from its being taken. Likewise, prior to analysis, these were thawed and centrifuged at room temperature at 1,000 g

for 2 minutes to obtain the clear saliva sample. Subsequently, the cortisol salivary levels were measured by chemiluminesence technology (IDS-iSYS Salivary Cortisol) according to the protocol for salivary samples.



Sampling schedule

Home baseline salivary sampling was carried out on a day without Zootherapy meeting at 6.30h. Saliva samples were taken from elderly (n=10) and Zootherapist (n=1). To lessen the effect of potential circadian deviation on salivary cortisol, before and after each meeting time, between 10.00 and 12:00 a.m., saliva samples were taken from elderly and Zootherapists.

This study was approved by the Ethics Committee of the University of Studies of Naples Federico II, and was in compliance with the Helsinki Declaration Provisions (1996). Informed consent was obtained from all participants. Sensitive data were handled with confidentiality and securely stored. Upon the Unit Management's approval and upon patients' informed consent, each Zootherapy meeting was filmed. Each video clip received supervision by the team.

Statistical analysis
Data were include in an Excel file. At each time (T_0 and T_1) the mean values and SD of salivary cortisol levels were calculated. Differences for Zootherapist Veterinary and elderly between T_0 and T_1 for salivary cortisol levels were analysed by using the Student's t-test for paired data. Statistical analysis was performed using SPSS 13 software for Windows (SPSS Inc., Chicago, IL, USA).

Results

A total of 220 salivary samples were collected from one Veterinary Zootherapist (n=20), and from 10 elderly (n=200). 11.3% samples yielded an insufficient amount of saliva and could not be used for analysis. Reasons for these failures included failure to chew the cotton pad. Moreover, occasionaly, elderly did not agree to sampling being taken.
Each single meeting showed a slight decrease in salivary cortisol between T_0 and T_1 of the elder, but only the average salivary cortisol means of the Veterinarian Zootherapist provided significant statistical results ($P<0.05$).

Discussion

As far as stress indices are concerned in healthy adults, previous studies showed a decrease in cortisol levels after interactions with dogs. The decline in cortisol levels was ascribed to a general de-arousal effect of affiliative animal-human interaction (Berry, 2012).
Our study, instead, even if it did not show significant reductions in statistical terms for each groups (Zootherapist/patient) at T_0 and T_1, for each person involved, it did show that the cortisol level reduction occurred even in the animal's absence since its protagonist role was only at a symbolic level and was evoked in each meeting. Moreover, the stress reduction found in the Zootherapist, despite its being minimal (2.81 ± 0.38 vs 1.24 ± 0.17) spurs us to reflect upon the fact that when suitably educated and trained, the professional figure may not only show a greater ability in flexibility and coping (Menna, 2016a), but is also much better equipped in handling the interspecific relationship communication even in the animal's absence.

Limitations

The main limitation of this study is the lack of a control group. Without the control group serving as a reference point, it is difficult to determine whether the results were solely due to our intervention or due to other factors. For example, the results can potentially be attributed to the fact that the elderly were very fond of animals and were dog lovers in particolar. All that we can say, at this point, is that there is no clear-cut certainty that results were caused by the Zootherapy intervention alone.

In addition, there are other factors that can influence cortisol responses that were not verified in this study. For example, the temperament, coping style and social competency of each partecipant (Curtis & Cicchetti, 2007; Gunnar, 2007).

Given the preliminary nature of this research; thus it will be important for future studies to confirm the findings by utilizing more rigorous plans that include larger sample sizes and random assignment to intervention and to control groups. In addition, future research will benefit from the inclusion of developmentally appropriate tests on emotional skills, such as emotion understanding and awareness.

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Chapter 7

7.1 Zoonoses and health protocols for co-therapist dogs involved in Zootherapy settings

In modern society traditional pets are often considered to be extended family. Having pets brings many benefits, such as psychological support, friendship, and even good health practices (exercising or stress reduction) (Overgaauw, 2009).

In comparison with food borne zoonoses, bacterial zoonoses associated with pets are still a relatively neglected area. In anyone household, however, the close pet-people contact is very conducive to transmission by contact. This may occur directly such as through petting, licking or physical injuries or indirectly through the contamination either of food or of the domestic premises. The importance of the role of contact is further shown by the frequent sharing of skin microbiota between people and their dogs (Song, 2013).

Zoonoses are of special concern in different age groups and in people who are particularly susceptible to infections.These include the young, the old, pregnant women or people who are immunocompromised. Moreover, bacteria originating from household pets may be particularly dangerous for young children due to their lower hygiene standards, having closer physical contact with pets and the household environment (such as floors and carpets) (Jacob & Lorber, 2015).

Infections with enteric bacteria and parasites pose the highest risk for human diseases from animals in public settings (LeJeune&Davis, 2004). Healthy animals can harbour human enteric pathogens, many of which have a low infectious dose (Chappell, 1996). Many pathogens have been responsible for outbreaks, including Escherichia coli O157:H7 and other Shiga toxin-producing *E. coli* (STEC), *Salmonella* enterica, *Cryptosporidium* species, and *Campylobacter* species (Shukla, 1995, Chapman, 2000). Although reports often document cattle, sheep, or goats (Steinmuller, 2006; CDC, 2004, Goode, 2009) as sources for infection, live poultry (CDC, 2006), and other domestic and wild animals also are potential sources.

The primary transmission mode for enteric pathogens is fecal-oral. Because animal fur, hair, skin, and saliva harbour fecal organisms, transmission can occur when people pet, touch or feed the animal or they get licked by them. Transmission also has been associated with contaminated animal bedding, flooring, barriers, other environmental surfaces, and contaminated clothing and shoes (Varma, 2003). Animals carrying human enteric pathogens fre-

quently show no signs of illness but can still shed the organisms, and thus contaminate the environment. In addition, treatment of animals can prolong shedding and contribute to antimicrobial resistance. Animals are more likely to shed pathogens due to stress induced by prolonged transport (Corrier, 1990) or restricted to small room areas, conditions which are quite frequent within AAIs contexts.

The risk for human infection is increased by certain factors and behaviours, especially in children and elderly. These factors and behaviours include lack of awareness of the risk for disease, inadequate hand washing, lack of close supervision, and hand-to-mouth activities (ex., thumb-sucking, and eating) (McMillian, 2007). Children are particularly attracted to animal venues but incur in higher risk for serious illness when they are infected.

Factors that increase risk include unsuited premises that may compromise the incoming and outgoing flow of pathogens.

Based on these premises, a Zootherapy setting, then, could possibly be a transmission system that is simultaneously dangerous and complex. In fact, if we take into consideration the Zootherapy setting as an Epidemiology Triad "agent - environment - host" (figure 7.1; 7.2), rather restrictive surveillance norms are planned for and put in place in the various therapeutic contexts. According to Federico II Model, these are classified as settings with a:

- *low surveillance level*: school areas and recreational areas
- *medium surveillance level*: areas not requiring full aseptic conditions (i.e. reception areas in medical structures, rehabilitation centres, some hospital wards
- *high surveillance level*: hospital wards with immunocompromised patients

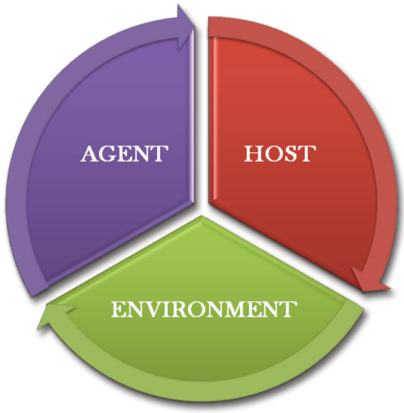

Fig. 7.1 Epidemiological Triade.

- ✓ The agent represents the "primary" determinant of an illness, necessary but not sufficient;
- ✓ the environment is made up by endogenous determinants (climate, microclimate, shelters, foods and so on);
- ✓ infection of the host depends on endogenous factors (sex, race, age, immune status and so on).

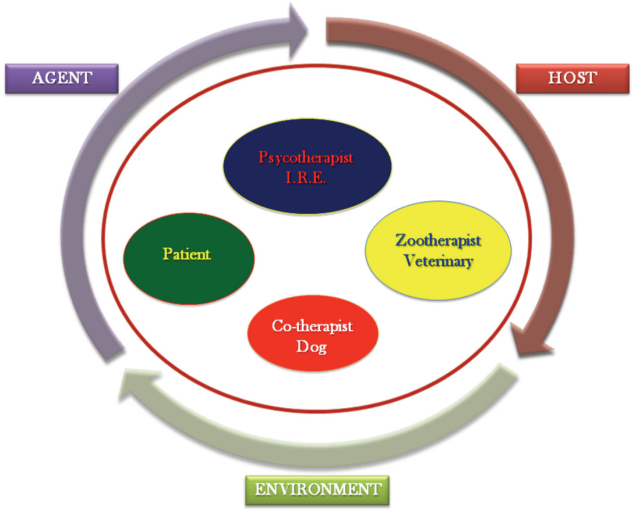

Fig. 7.1 Zootherapy setting and Epidemiological Triade: a compariso .

Although eliminating all risks from animal contacts is quite an improbable task, our research study provides recommendations for minimizing risks associated with the human-animal relationship within AAI contexts and in public settings. On the basis of these premises, we conducted 3 different survey. These were aimed at monitoring zoonotic agents such as *Pasteurella* spp, Thermotolerant *Campylobacter*, *Giardia duodenalis* and other endoparasites in dogs frequenting a Dog Educational Centre where dog-dog contact and dog-human contact were more frequent and more indiscriminate and thus, there was a risk of transmission of zoonotic agents. Furthermore, it is important to highlight that these surveys also included the co-therapist dogs involved in our Zootherapy programs (n=6) and all dogs tested were negative.

7.2 Epidemiological survey of *Pasteurella multocida* from oral cavity of dogs

Several reports describe human infections by *Pasteurella* spp. after contact with pets, including sharing a bed, being licked by, or kissing the pets (Kawashima, 2010; Chomel BB, 2011). In humans, *P. multocida* can cause serious infections both invasive and localized into the oral cavity, the respiratory system and soft tissue (Miyoshi, 2012). Zoonotic transmission usually occurs by contact of skin lesions or through animal nasal secretions, particularly as this pertains to young children, elderly, or pregnant or immunocompromised individuals (Wilson, 2013). *Pasteurella multocida* is a non motile, non sporige-forming, gram-negative coccobacillus, which belongs to the oral flora of most mammals, including cats and dogs (Guay DR., 2001).

The present study was carried out to evaluate the prevalence of *P. multocida* in oral cavity of dogs in a Dog Educational Centre (DEC), where dog owners and/or other family members spend time on a weekly and/or daily basis for canine education courses, agility trials, training and interaction with other dogs, puppy classes and kindergarten for dogs. In a DEC, both dog-dog contact and dog-human contact are more frequent and more indiscriminate; thus, the risk of transmission of zoonotic agents as *Pasteurella multocida* may increase.

Our study was performed from May to November 2014 on 160 dogs frequenting a DEC in Southern Italy (Campania region). Each dog was individually sampled using oral swabs. The dogs data were collected using a brief questionnaire containing some generic characteristics (age, sex, breed, and habitat) and several questions regarding the health status. Dogs were classified in two *Age groups*, one containing animals from three to twelve months (n = 24) and the other containing animals over twelve months (n = 136); two *Sex groups*: male (n = 100) and female (n = 60); two *Breed groups*: crossbreed (n = 88) and purebred (n = 72); two *Life habit groups*: indoor (n = 136) and outdoor (n = 24). All the dogs sampled were apparently in healthy body conditions.

The animals examined in the present study were sampled following approval by the Animal Ethics and Welfare Committee of the University of Naples Federico II (Protocol Number 20412/ February 28th, 2014).

Materials and Methods

Isolation of bacteria

Oral swab samples were inoculated on to blood agar supplemented with 2 µg/mL clindamycin and incubated aerobically at 37 °C for 24 h. All catalase-positive, oxidase-positive, and small Gram-negative rods or coccobacilli observed under the light microscope were subcultured. Identification of *P. multocida* was performed biochemically (Forsblom B. et al, 2002; Dousse F. et al, 2008).

Polymerase chain reaction (PCR)

One or two isolated colonies were used for DNA extraction using the Prep-Man sample reagent (PE Applied Biosystems), following the manufacturer's protocol. For *P. multocida* specific PCR were used a pair of primers (Eurofins), KMTJB-for TGCCACTTGAAATGGGAAATG and KMTJB-rev AATAACGTCCAATCAGTTGCG, obtained by searching all DNA sequences of *P. multocida* (available in GenBank) encoding the outer-membrane protein (KMT-1) (Jaroslaw Krol et al., 2011). The reaction mixture (50 µL) contained 2 µL of mix oligos, 25 µL of MyTaq Red Mix (Bioline), 2 µL di DNA. Forty PCR cycles of denaturation at 95 °C for 1min, annealing at 57 °C for 15 s, and elongation at 72 °C for 10 min were performed in a Model 9600 thermal cycler (PE Applied Biosystems). The PCR products were separated by electrophoresis on 1.8% agarose gel (Gibco-BRL), stained with ethidium bromide and visualized under UV light and results were recorded using a ChemiImager 5500 (BSI). PCR amplified without the DNA was used as negative control, whereas reference *P. multocida* strains ATCC 43137 were used as positive controls (LGC Promochem).

Data analysis

Statistical analyses were performed using SPSS 20 Software for Windows. The dogs data (age, sex, bred and life habits) were analysed by univariate (Pearson's χ^2 test for independence) using the *Pasteurella multocida* status (positive⁄negative) as dependent variable. Only the independent variables that showed significance (P <0.01) in the univariate test were used for the logistic regression model (Hosmer & Lemeshow, 2000).

Results and Discussion

Out of 160 dogs examined, 20 [12.5%; 95% confidence interval (CI) = 8.0–18.9%] were positive for *Pasteurella multocida* by cultural and biochemical methods. As shown by PCR, all positive samples were confirmed as P. multocida (table 7.1). In particular, female dogs showed a high prevalence of *P. multocida,* at 23.3%, whereas male dogs showed a prevalence of 6.0%. This difference was statistically significant (P <0.01). Moreover, crossbred dogs showed a prevalence of 18.2% (95% CI = 13.7–23.7%) for *P. multocida*, whereas purebred dogs showed a prevalence of 5.6% (95% CI = 3.1–10.0%); this difference was statistically significant (P <0.01). Finally, dogs living outdoor showed a prevalence of 41.7% (95% CI = 26.9–58.1%) for *P. multocida*, whereas dogs living indoor showed a prevalence of 7.3% (95% CI = 6.2–9.4%); this difference was statistically significant (P <0.01). In contrast, there was no significant difference related to age (P >0.05). With respect to the statistical regression model results, sex and life habit were risk factors for *P. multocida* positivity (table 7.2).

It is difficult to speculate on the higher prevalence of P. multocida in female dogs, but we hypothesise that it could be connected to different hormonal states, but further investigation is needed. In addition, with regard to the higher prevalence of *P. multocida* in dogs living outdoor, we hypothesize that frequent contact with excreta of other animals reservoir (birds, rodents, other wild mammals) could increase a contamination risk for dogs. Since dogs are a potential source of *P. multocida* infection for people, it is important to minimize the risk of zoonotic spread by encouraging humans to follow good hygiene practices and to emphasise the health check requirements for owning a dog, particularly when a place such as a DEC is frequented daily by multiple dogs as well as operators, Veterinarians, dog owners, dog trainers, children and visitors.

Although further studies are needed to understand the mechanisms underlying the variable-related differences reported in the present study, to our knowledge, this is the first study on the prevalence of *P. multocida* in dogs attending a Dog Educational Centre.

Tab. 7.1 Dog data and positivity to Pasteurella multocida.

Dogs data	Number of samples	Number of samples positive for *P. multocida* (%positive)[a]	CI* (95%)	*P***
Sex				
Male	100	6 (6.0)	3.7 - 9.4	0.001
Female	60	14 (23.3)	16.7 - 31.9	
Bred				
Crossbred	88	16 (18.2)	13.7 - 23.7	0.01
Purebred	72	4 (5.6)	3.1 - 10.0	
Life habit				
Outdoor	24	10 (41.7)	26.9 - 58.1	0.000
Indoor	136	10 (7.3)	6.2 - 9.4	

*CI 95%, Confidence Interval (95%); **P, χ^2.

Tab. 7.2 Dog data and positivity to Pasteurella multocida.

Independent variable	Standard error	*P* value	Odds ratio	95% confidence interval Low
Sex				
Female vs Male	0.57	0.01	0.25	0.08
Breed				
Crossbred *vs* Purebred	0.63	0.08	3.02	0.9
Life habit				
Outdoor *vs* indoor	0.58	0.00	0.12	0.03

Dependent variable is Pasteurella multocida positivity.

7.3 Epidemiological survey of *Campylobacter* thermotolerant in dogs at Dog Educational Centres

Campylobacter species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are considered to be the most frequent bacterial cause of acute human gastroenteritis in many industrialized countries (Dipineto *et al.* 2008). *C. jejuni* can be a commensal in the intestinal tract of many mammals and several avian species (Dipineto *et al.* 2014; Gargiulo *et al.* 2008; Santaniello *et al.* 2014). The main risk factors for humans include the consumption of contaminated food and drinking water, but direct contact with carrier animals was also found to be a possible source of infection for *C. jejuni* (Mughini Gras *et al.* 2013; Ramonaite *et al.* 2014; Wieland *et al.* 2005). *C. lari* and *C. upsaliensis* are rarely found in humans, and their occurrence may vary among different regions of the world (Carbonero *et al.* 2012; Moore *et al.* 2005; Wieland *et al.* 2005). Dogs can be healthy carriers of *Campylobacter* spp., and animals under six months of age show a higher rate of carriage. Higher carriage rates were also observed in younger dogs with diarrhoea, while in older dogs, no difference in *Campylobacter* spp. shedding was reported between healthy and diseased animals (Burnens *et al.* 1992).
Although there have been several studies on the presence of *Campylobacter thermotolerant* in dogs (Amar *et al.* 2014; Andrzejewska *et al.* 2013; Badlìk *et al.* 2014; Carbonero *et al.* 2012), no previous investigations in Dog Educational Centres (DEC) have been conducted. Dog owners and their family members go to these centres weekly and/or daily basis for canine education courses, agility trials, training and interaction with other dogs, puppy classes and kindergarten for dogs. Both dog-dog contact and dog-human contact are more frequent and more indiscriminate; thus, the risk of transmission of zoonotic agents such as *Campylobacter* spp. may increase. Therefore, the aim of our study was to evaluate the presence of *Campylobacter* spp. in dogs frequenting DECs in Southern Italy.

Materials and Methods

Sampling
Our study was held between October 2015 and May 2015 in five DECs in Campania region (Southern Italy). Rectal swab samples were collected from a total of 550 dogs. This sample size was calculated using the formula pro-

posed by Thrusfield (1995) for a large (theoretically 'infinite') population using the following values: expected prevalence (8.0%), confidence interval (95%) and desired absolute precision (5%). The DECs were identified as DEC1, DEC2, DEC3, DEC4 and DEC5, in which 180, 112, 180, 43, and 35 dogs were sampled, respectively. Each dog was individually sampled using rectal swabs. The information for each dog was collected through an interview performed on arrival at the DEC by researchers of the working group using a semi-structured questionnaire addressing some generic characteristics (age, sex, breed, and eating habit) and different questions regarding health status. The dogs were classified into two *Age groups*, one containing animals from three to six months of age (n = 245) and the other containing animals over six months (n = 305); two *Sex groups*, male (n = 299) and female (n = 251); two *Breed groups*, crossbred (n = 385) and purebred (n = 165); three *Eating habit groups*: dry food (n = 378), canned meat (n = 154) and home-cooked food (n = 18).
All the dogs sampled were apparently in good health.
This study was approved by the Animal Ethics and Welfare Committee of the University of Naples Federico II (Protocol Number 16683/June 13th, 2013).

Isolation of bacteria
The rectal swab samples were stored in Amies Transport Medium (Oxoid, Basingstoke, UK) at +4°C, transported to the laboratory and analysed within 2h of collection. Samples were inoculated into *Campylobacter*-selective enrichment broth (Oxoid) and incubated at 42 °C for 48h under microaerobic conditions provided by CampyGen (Oxoid). Subsequently, each sample was streaked onto *Campylobacter* blood-free selective agar (CCDA; Oxoid) with the corresponding supplement (SE 155; Oxoid). After incubation at 42 °C for 48 hrs under microaerobic conditions, the plates were examined for typical *Campylobacter* colonies. The suspected colonies were purified on sheep blood agar (SBA; Oxoid) and finally incubated for 24 hrs at 42 °C. Colonies comprising curved or spiral motile rods, when observed under phase contrast microscopy, were presumptively identified as *Campylobacter* and identified at the species level by reaction to Gram staining, oxidase, catalase and hippurate tests as well as susceptibility to nalidixic acid and to cephalothin, according to the International Standard Procedures (ISO 10272 1995).

Polymerase chain reaction (PCR)
The extraction and purification of DNA from isolated colonies on sheep blood

agar was performed using a Bactozol kit (Molecular Research Centre, Inc., Cincinnati, OH, USA) as described previously (Khan and Edge 2007). The specific detection of the *Campylobacter* genus was based on PCR amplification of the cadF gene using the oligonucleotide primers cadF2B and cadR1B, as described by Santaniello et al. (2014).
All DNA extracts were also examined by triplex PCR for the presence of *C. jejuni*, *C. coli* and *C. lari* species using amplification conditions and the oligonucleotide primers ICJ-UP and ICJ-DN, ICC-UP and ICC-DN, and ICL-UP and ICL-DN, respectively, as previously described (Khan and Edge 2007). PCR products were separated by electrophoresis on 1.5% agarose gels (Gibco–BRL, Milan, Italy), stained with ethidium bromide and visualized under UV light. PCR amplified without the DNA template was used as negative control, whereas three reference *Campylobacter* strains, *C. jejuni* ATCC 29428, *C. coli* ATCC 33559 and *C. lari* ATCC 43675, obtained from LGC Promochem (LGC Promochem, Teddington, UK), were used as positive controls.

Data analysis
All the statistical analyses were performed using SPSS 20 Software for Windows. Data were recorded in an Excel file. The dog data (age, sex, bred and usual food) underwent univariate analysis (Pearson's chi-square test for independence) using the *C. jejuni* and *C. coli* status (positive/negative) as dependent variables. Only the independent variables that showed significant differences ($P < 0.05$) in the univariate test were used for the logistic regression model. If interaction between variables was suspected, a logistic regression model was run with and without these variables to evaluate possible effect modification (Hosmer&Lemeshow 2000).

Results and Discussion

Out of 550 dogs examined, 135 [24.5%; 95% confidence interval (CI) = 21.0 – 28.4%] were positive for *Campylobacter* spp. As shown by PCR, 84/135 (62.2%, CI = 53.4 – 70.3%) positive samples were identified as *C. jejuni*, whereas 51/135 (37.8, CI = 29.7 – 46.6%) positive samples were identified as *C. coli.* In contrast, *C. lari* was not isolated and *C. upsaliensis* was not detected, as all strains isolated by the culture methods were negative for the catalase test. In particular, dogs fed with home-cooked food showed a high prevalence

of *C. jejuni,* at 50.0%, whereas dogs fed with dry food and canned meat showed a prevalence of 14.3% and 13.7%, respectively. These differences were statistically significant ($P < 0.01$), as shown in table 1. Moreover, purebred dogs showed a prevalence of 16.4% (95% CI = 11.2 – 23.1%) for *C. coli*, whereas crossbred dogs showed a prevalence of 6.2% (95% CI = 4.1 – 9.3%); this difference was statistically significant ($P < 0.01$), as shown in table 2. In contrast, there was no significant difference related to age and sex ($P < 0.05$). With respect to the statistical regression model results, breed and eating habit were risk factors for *C. coli* and *C. jejuni* positivity, respectively. Specifically, purebred dogs had a significantly higher risk of being positive for *C. coli* than crossbred [odds ratio (OR) = 2.042; $P < 0.01$). Dogs fed with home-cooked food had a significantly higher risk of carrying *C. jejuni* than dogs fed with canned meat (OR = 4.766; $P = 0.002$) and dogs fed with dry food (OR = 3.831; $P = 0.006$). All results of the logistic regression model are listed in table 7.5. Given that the five DECs examined differed in management and geographic location and were sampled at different times, statistical analysis within each DEC was conducted, considering the same group categories (age, sex, breed and usual food).

In DEC1, out of the 180 dogs examined, a total of 34 (18.9%; 95% CI = 13.6 – 25.5%) were positive for *Campylobacter* spp. As determined by PCR, 24 (13.3%) were positive for *C. jejuni* and 10 (5.5%) for *C. coli*. Purebred dogs (35.0%) showed a *C. jejuni* prevalence of 20.6% (95% CI = 11.9 – 33.0%), whereas crossbred dogs (65.0%) showed a prevalence of 9.4% (95% CI = 5.0 – 16.6%); this difference was statistically significant ($P < 0.05$).

In DEC2, out of the 112 dogs examined, a total of 21 (18.7%; 95% CI = 12.2 – 27.5%) were positive for *Campylobacter* spp. As determined by PCR, 13 (11.6%) were positive for *C. jejuni* and 10 (8.9%) for *C. coli,* but 2 dogs were positive for *C. jejuni* and *C. coli* at the same time. Purebred dogs (32.1%) showed a *C. jejuni* prevalence of 22.2% (95% CI = 10.7 – 39.6%), whereas crossbred dogs (67.8%) showed a prevalence of 6.6% (95% CI = 2.4 – 15.3%); this difference was statistically significant ($P < 0.05$). In addition, purebred dogs showed a *C. coli* prevalence of 19.4% (95% CI = 8.8 – 36.6%), whereas crossbred dogs showed a prevalence of 3.9% (95% CI = 1.0 – 11.9%); this difference was statistically significant ($P < 0.01$).

In DEC3, out of the 180 dogs examined, a total of 46 (25.6%; 95% CI = 19.5–32.7%) were positive for *Campylobacter* spp. As determined by PCR, 26 (14.44%) were positive for *C. jejuni* and 20 (11.11%) for *C. coli*. Purebred

dogs (24.4%) showed a *C. coli* prevalence of 22.7% (95% CI = 12.0 – 38.2%), while crossbred dogs (75.5%) showed a prevalence of 11.03% (95% CI = 6.51 – 17.83%); this difference was statistically significant ($P < 0.05$). In this DEC, the 92 dogs under six months of age showed a higher prevalence of *C. coli* (19.3, 95% CI = 12.0 – 29.4%) compared to the 88 dogs older than six months, and this difference was statistically significant. In addition, although these results were conditioned by the small size of the samples, the dogs fed home-cooked food showed a very high prevalence of *C. jejuni* (100.%; 95% CI = 31.0 – 96.8), with a statistically significant difference compared to dogs fed with dry food and canned meat ($P < 0.05$).

In DEC4, out of the 35 dogs examined, a total of 21 (60.0%; 95% CI = 42.2 – 75.6%) were positive for *Campylobacter* spp. As determined by PCR, 12 (34.3%) were positive for *C. jejuni* and 6 (17.1%) for *C. coli*. The data from this DEC showed no statistically significant differences.

In DEC5, out of the 43 dogs examined, a total of 46 (25.6%; 95% CI = 19.5 – 32.7%) were positive for *Campylobacter* spp. As determined by PCR, 10 (21.7%) were positive for *C. jejuni* and 6 (13.9%) for *C. coli*. In this DEC, the 18 dogs under six months of age showed a higher prevalence of *C. jejuni* (66.7, 95% CI = 41.1 – 85.6%) than the 25 dogs older than six months, and the difference was statistically significant.

The findings of this survey demonstrate the occurrence of *C. jejuni* and *C. coli* in dogs at all five DECs in Southern Italy, with a prevalence of 15.3% for *C. jejuni* and 9.3% for *C. coli*, whereas *C. lari* was not isolated. However, our results showed the highest prevalence among data from the recent scientific literature in Europe and in Italy on the prevalence of thermotolerant *Campylobacter* in dogs. In fact, in Wieland *et al.* (2005) isolated *C. jejuni* with a prevalence of 5.7%, *C. coli* with a prevalence of 1.1%, *C. lari* with a prevalence of 0.9% and *C. upsaliensis*/*C. helveticus* with a prevalence of 30.4%, whereas in Norway, Sandberg et al. (2002) isolated *C. jejuni* with a prevalence of 3.0% and *C. upsaliensis* with a prevalence of 2.·0%. In Poland, Andrzejewska et al. (2013) conducted a survey in 83 dogs (and 71 cats) and isolated *Campylobacter* spp. with a prevalence of 4.8%, of which *C. jejuni* was the predominant species. In Slovakia, Badlìk et al. (2014) isolated *Campylobacter* spp. with an overall prevalence of 30.4% and *C. jejuni* and *C. coli* with prevalences of 51.2% and 9.8%, respectively. In Italy, Rossi et al. (2008) conducted a survey in dogs and in cats, isolating *Campylobacter jejuni* in 8.9% of 190 dogs sampled.

Although it was not possible to speculate on the highest prevalence of *C. coli* in purebred dogs, we hypothesise that the strong selection for morphological characteristics in these animals may influence resistance to infection. In addition, with regard to the heightened prevalence of *C. jejuni* in dogs fed with home-cooked food, we hypothesize that the preparation of the food (commercial dry food, commercial canned meat and home-cooked food prepared from fresh foods of animal origin) could be a risk factor to be monitored not only for animal health status but also for public health.

Dogs are a potential source of *C. jejuni* and *C. coli* infection for people, and given that they cohabit with humans in places such as parks, public gardens, and squares, it is important to minimize the risk of zoonotic spread by encouraging humans to follow good hygiene practices and to emphasise the health check requirements for owning a dog, particularly when a place such as a DEC is frequented daily by multiple dogs as well as operators, Veterinarians, dog owners, dog trainers, children and visitors. In addition, a DEC has an outdoor area for activities, which may also be accessed by many species of birds, wild mammals and/or sinanthropus. Thus, it is important to increase attention to the role of the latter as vectors of contamination "upstream or downstream" for *Campylobacter* spp.

Tab. 7.3 Dog data and positivity for Campylobacter jejuni.

Dog data	No. tested dogs	*No. positive dogs*	%	95% CI	*P**
Age					
< 6 months	245	41	16.7	12.4 - 22.1	0.393
> 6 months	305	43	14.1	10.5 - 18.6	
Sex					
Male	299	42	14.0	10.4 - 18.6	0.383
Female	251	42	16.7	12.4 - 22.1	

continued

Dog data	No. tested dogs	*No. positive dogs*	%	95% CI	*P**
Bred					
Crossbred	385	53	13.8	10.6 - 17.7	0.134
Purebred	165	31	18.8	13.3 - 25.8	
Eating habit					
Dry food	378	54	14.3	11.0 - 18.3	0.000
Canned meat	154	21	13.6	8.8 - 20.3	
Home-cooked	18	9	50.0	26.8 - 73.2	
Total	550	84	15.27	12.42 - 18.62	

CI, confidence interval; *Chi-square.

Tab. 7.4 Dog data and positivity for Campylobacter coli.

Dog data	No. tested dogs	*No. positive dogs*	%	95% CI	*P**
Age					
< 6 months	245	26	10.6	7.2 - 15.3	0.332
> 6 months	305	25	8.2	5.5 - 12.0	
Sex					
Male	299	30	10.0	7.0 - 14.1	0.502
Female	251	21	8.4	5.4 - 12.7	
Bred					
Crossbred	385	24	6.2	4.1 - 9.3	0.000
Purebred	165	27	16.4	11.2 - 23.1	
Eating habit					
Dry food	378	39	10.3	7.5 - 13.9	0.446
Canned meat	154	11	7.1	3.8 - 12.7	
Home-cooked	18	1	5.6	0.3 - 29.4	
Total	550	51	9.3	7.0 - 12.1	

CI, confidence interval; *Chi-square.

Tab. 7.5 Results of logistic regression model.

Independent variable	Standard error	*P* value	Odds ratio	95% confidence interval	
				Low	*High*
Breed* Purebred vs Crossbred	0.210	0.001	2.042	1.352	3.084
Eating habit** Dry food *vs* Canned meat	0.232	0.346	0.804	0.510	1.266
Dry food *vs* Home-cooked food	0.489	0.006	3.831	1.469	9.992
Canned meat *vs* Home-cooked food	0.514	0.002	4.766	1.739	13.057

Dependent variable is *Campylobacter jejuni*** or *Campylobacter coli** positivity.

7.4 Parasitic infections in dogs involved in Animal Assisted Interventions

In order to control zoonotic diseases naturally transmitted between vertebrate animals and humans, in 2004, the international scientific community used the term "One Health" to define the need for a multidisciplinary approach including human and veterinary medicine, and environmental sciences in Public Health (Chalmers & Dell 2015). Animal Assisted Interventions (AAIs) programs represent current and concrete examples of One Health, as they involve many health care figures who work in team for the welfare of people, considering the animal (i.e. a co-therapist dog) as a referent of the therapeutic process (Menna, 2016a). Indeed, the human-animal interactions, particularly with dogs, have been associated with positive effects on human health and wellbeing (Fine, 2010; Menna, 2012). The AAIs approach includes animal-assisted therapy (AAT), animal-assisted activities (AAA) and animal-assisted education (AAE); these three activities can produce therapeutic, motivational and educational benefits in people of all ages, particularly among children and the elderly, and in different kinds of patients, from those with physical ailments to those with mental disorders such as dementia and depression (Menna, 2016b; Pedersen, 2011). Consequently, AAIs programs have been considered useful in different settings, such as hospital, therapeutic, educational and assisted living environments (Reed et al. 2012; Banks & Banks 2002), hence becoming very important in public health care system as a complementary intervention to conventional therapies and activities. In this context, it should be noted that wherever there are animals not subjected to appropriate health control, there could be a serious risk of transmission of zoonotic (infective or parasitic) agents.

Domestic dogs are important reservoirs of many zoonotic pathogens including several gastrointestinal parasites (Paul et al. 2010; Robertson & Thompson 2002) The parasitic risks for humans are mostly posed by environmental fecal contamination (Rinaldi et al. 2006). The presence of eggs on the ground is not only implicated with the direct infection for humans but could represent a source of contaminations for pet coats. Indeed, some studies confirmed that the presence of parasitic elements (e.g., embryonated ascarid eggs or *Giardia* cysts) on the fur of dogs has been evocated as a source of human infections via hand-to-mouth contact (Traversa et al. 2014). Eggs, larvae, cysts, and oocysts excreted via the canine faecal route can survive and be infective in the environment over a long time and under different conditions (Rinaldi et al. 2006). Dog faeces deposited on soil in city parks or gardens represent not

only an inconvenience, but also can be a health threat (Traversa et al. 2014). Although the canine geohelminths (*Toxocara canis, Ancylostoma caninum* and *Trichuris vulpis*) are well-known recognized zoonotic parasites, in recent years major attention have been paid to the protozoan *Giardia duodenalis* which is now considered the intestinal parasite with a high zoonotic potential among domestic carnivores (Macpherson, 2013; Ryan & Cacciò 2013; Zanzani et al. 2014b) as well as the leading cause of parasitic gastroenteritis worldwide (Minetti et al. 2015). One of the limits of research about *G. duodenalis* in humans is the entity of the risk, in fact, still now, it is not well known (Bouzid et al. 2015). The situation in dogs is more clear with prevalence of *G. duodenalis* ranging from 1.3% to 24.8% (Epe et al. 2010; Zanzani et al. 2014a). Therefore, the aim of the present study was to investigate, for the first time in Italy, the presence of *G. duodenalis* and zoonotic gastrointestinal nematodes in dogs involved in AAIs.

Materials and methods

Study area

The study was conducted in a dog educational centre in the city of Naples (southern Italy) where the animals were trained to AAIs through an educational program according to the guidelines of the Italian National Educational Sports Centre (CSEN). From April to June 2016, a total of 74 faecal samples was collected from owned dogs used in AAI. The information for each dog was collected through an interview performed on arrival at the Centre using a semi-structured questionnaire addressing some generic characteristics (age, sex, and breed of the dogs) and different questions regarding their health status. The dogs were classified into two Age groups, one containing animals from two to five years (n =38) and an other containing animals from six to ten years (n =36); two *Sex groups*, male (n =32) and female (n =42); two *Breed groups*, crossbred (n = 36) and purebred (n =38). All the animals were natives from the Campania region of southern Italy. Which extends over an area of 13,590 km² is mainly hilly and extends from 0 to 1,890 m above sea level. The climate is Mediterranean with dry summers and rainy winters.

Faecal sampling and FLOTAC technique

Two grams of faeces were placed into the Fill-FLOTAC (Cringoli et al. 2013), a plastic kit to weight, dilute, homogenize and filter the sample. Once in the lab,

18 ml of water (dilution ratio = 1:10) were added to the fresh faeces contained in the Fill-FLOTAC. The suspension was then thoroughly homogenized using the homogenizer stick of the Fill-FLOTAC. The faecal suspension was filtered through the Fill-FLOTAC and 6 ml of the filtered suspension were placed and centrifuged into two conic tubes. After centrifugation (3 minutes at 1500 rpm) the supernatant was discarded leaving only the sediment (pellet) in the tube.
Copromicroscopic examinations were performed using the FLOTAC dual technique (Cringoli et al. 2010) for the detection of helminth eggs and protozoan cysts. This technique is based on the use of two flotation solutions: Sodium Chloride (s.g.= 1200) and Zinc Sulfate (specific gravity - s.g. = 1200). The analytic sensitivity of the FLOTAC dual technique was 2 cysts/eggs per gram (CPG/EPG) of faeces (Cringoli et al., 2010).

Results

Out of the 74 faecal samples examined during the study, 18 were positive for any parasitic element (24.3%; 95% CI= 15.4-35.9) (Table 1). The higher prevalence values were found for *Giardia* (10.8%) and *T. vulpis* (9.5%). Some coinfections were found during the copromicroscopic investigation: specifically, one sample was positive to both *T. canis* and *T. vulpis* (1.4%; 95% CI= 0.1-8.3) and two samples were positive to both *G. duodenalis* and Ancylostomidae (2.7%; 95% CI= 0.5-10.3)

Discussion

This is one of the first parasitological studies in Italy in dogs that participate in Animal Assisted Interventions. The presence of four zoonotic parasites (nematodes and protozoa) in these animals, suggest that dogs involved in AAIs could play an important epidemiological role in the transmission of parasitic infections to humans.
Dogs that participate in AAIs, commonly interact with humans whose immune systems are not functioning optimally (Lefebvre, 2008; Kamioka, 2014) and several studies show that chronic *Giardia* infection occur in children and immunocompromised individuals (Robertson, 2010; Thompson, 2004).
The dog is one of the pet closest to the human and the transmission of zoonotic agents is also favoured given the close relationship of human beings with their pet (Feng & Xiao 2011).

According to Bouzid et al. (2015) what risk such endemic colonisation poses to human health is still unclear as it will depend not only on prevalence rates but also on what assemblages of *Giardia* are excreted and how people interact with their pets. In fact, the level of risk depends strictly on the presence of *Giardia*-human assemblages (A and B) (Cacciò. 2005). In addition, recent data show the case of a dog that has been associated exclusively to the A assemblage, suggesting the existence of a potential zoonotic reservoir for this assembly (Minetti et al. 2015). Unfortunately, one of the limits of our study is that assemblages of *G. duodenalis* have not been determined by molecular investigations.

Considering that children, young adults and immunocompromised individuals are among the main users of the AAIs, the animals involved should also undergo a specific parasitological diagnosis targeting *Giardia* spp. and other intestinal nematodes (Lefebvre 2008; Silveira 2011). It would be useful to control and reduce the presence of these parasites, also preventing the human transmission where there is an increased risk of infections, such as in childcare centers and day-care centers (Cordell, 2001). Therefore, monitoring dogs for zoonotic parasites is necessary for the development and implementation of effective control and prevention strategies that mitigate the burden of zoonotic diseases on Public Health. Prevention through specific guidelines including suitability of patients, animals and infection control policies, need to be formulated before the initiation of AAIs and this is the most important way to avoid human dog infections.

Tab. 7.6 Parasitological results in dogs involved in Animal Assisted Interventions in southern Italy.

Parasites	No. of positive dogs = 18			
	No. of positive	Prevalence (%)	95% Confidence Interval	CPG/EPG* (min-max)
Giardia duodenalis	8	44.4	22.4-68.7	10-30,000
Trichuris vulpis	7	38.9	18.3-63.9	2-154
Toxocara canis	3	16.7	4.4-42.3	50-1260
Ancylostomidae	1	5.6	0.3-29.4	32

CPG/EPG = cysts/eggs per gram of faeces

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Chapter 8

8.1 Final discussion and conclusion

The key focus and strength of our research study revolves around the investigation conducted on the therapeutic effects of Zootherapy. Yet, we dare to say that our study is among the first at international level with regards to the process analysis of Animal-Assisted Interventions (AAIs) as it expresses itself through the scientific premises, the intervention protocols and through the results that were obtained by theorizing and by applying the Federico II Model of Zootherapy.

Veterinary Medicine across the world plays an important social role in safeguarding animal and Public Health (Tannenbaum, 1995; Yeates, 2010). The veterinary profession has many roles and responsabilities in its relationship with other animals. The challenge for this profession today is to find ways of integrating into veterinary training both traditional competencies such as knowledge in animal management, husbandry, behavior, genetics, housing, nutrition and welfare with needs that have risen as a consequence of modern living which are mainly represented by Animal-Assisted Interventions.

As a result of the need to evaluate the person in its complexity, in the context of human and veterinary medicine, there has been a growing interest in integrated approaches whose aim is prevention, diagnosis and treatment of certain diseases. More specifically, the need to develop new policy approaches that can also intervene on environmental and psychosocial factors and on human lifestyle has led to the creation of *Healthcare Zooanthropology*, understood as the study, administration and application of the relationship between humans and animals in contexts that are both healthcare/therapeutic and didactic which can be an effective resource for health promotion. As a result, the AAIs are a type of therapeutic work that must be classified as part of health services rendered just as intervention must be accepted as operating within the field of human health. It becomes clear, therefore, that *Healthcare Zooanthropology* is a new field of study and a potential professional category for Veterinarians. This is evidenced by Animal Assisted Interventions which assign pets with an important role in maintaining and improving human health.

Today, dogs continue to play a major role in the lives of people around the world. Moreover, although animal contact carries risks, the frequency of most zoonotic diseases can be lessened, perhaps even eliminated, with animal management practices that would serve humans and animals alike. Veterinary

care to manage bacterial, viral, and parasitic infections, responsible legislation, owner education (Macpherson, 2013) as well as the presence in a setting of a Veterinary Zootherapist as *tutor* and handler of the animal could very well turn the relationship with the dog into a safe, healthy, and rewarding experience for all involved (Menna, 2016a).

The One Health concept is a global strategy to expand interdisciplinary collaborations and communications in all aspects of health care for humans, animals, and the environment. Recognizing that human health (including mental health via the human–animal bond phenomenon), animal health, and ecosystem health are inextricably linked, One Health seeks to promote, improve, and defend the health and well-being of all species by enhancing cooperation and collaboration amongst physicians, veterinarians, and other scientific health and environmental professionals.

The One Health intentions and their realization policy cannot dispense from under stand the need to define new training contexts as well as new professional figures. Consequently, we hold that the role of the Veterinary Zootherapist represents a new social resource just as *Healthcare Zooanthropology* is a new trans-disciplinary field, so as to safeguard the integrity of the ecosystem.

8.2 References

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