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Course
Human and non-human animals interaction: contextual, normative and applicative aspects

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The Course has been addressed to medical doctors, veterinarians and animal care personnel. The broad aims of the meeting have been to investigate and discuss the different kinds of human-animal relationship and to provide knowledge and an updating of the rules on the protection of animals. These kinds of courses adhere to the goals the National Health Program, due to the widespread presence of animals in the modern community which forces to deal with the aspects and problems linked to this interaction. The main issues considered have been the relationship with pets, animal experimentation, pet therapy and breeding of farm animals. In particular, the present Italian legislation on the use of animals for scientific research, the European Directive 86/609 and Regional Agreement for the Pet therapy 6/2/2003 have been discussed as well.
Key words: Animal welfare, Legislation, Bioethic

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Corso. Uomini e animali, differenti contesti e modalità di interazione: aspetti applicativi e normativi. Istituto Superiore di Sanità. 18-19 dicembre 2006.
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Il Corso, rivolto a medici chirurghi e medici veterinari, ha trattato le tematiche relative al rapporto essere umano e animali. Obiettivo del Corso è stato quello di affrontare la relazione essere umano-animale nei differenti contesti e di aggiornare il personale operante sulle vigenti norme e i recenti progressi in materia di utilizzazione degli animali. Tale Corso è rientrato negli obiettivi del Piano Sanitario Nazionale, poiché la presenza diffusa degli animali nella vita sociale ed economica impone la necessità di approfondire gli aspetti e le problematiche derivanti da tale interazione. I temi trattati hanno riguardato il rapporto con gli animali d'affezione, l'utilizzo degli animali nella sperimentazione biomedica, la loro presenza in ambito sanitario attraverso la *pet therapy* e l'allevamento degli animali da reddito. In ambito normativo, sono state trattate le tematiche relative alla legislazione vigente, con particolare riferimento alla Direttiva 116/1992 e all'Accordo Stato-Regioni del 6/2/2003 in materia di *pet therapy*.
Parole chiave: Benessere animale, Legislazione, Bioetica

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TABLE OF CONTENTS

INTRODUCTION

Humans have a long history of special bond with animals. In fact, from earliest cultures, animals have played an important role in our life, for example, through practices such as the domestication. Initially animals have been used in agriculture and for the production of meat and milk and then, subsequently, have been playing more different. Today, in the modern community, the animals, besides to remain an essential part of intensive food production, have a dominant role as companionship. The family pets, in particular dogs and cats, provide certain components of attachment that contribute to humans' emotional and social well-being throughout the life cycle. Furthermore, the animals are employed in scientific experiments to clarify the mechanisms of numerous human and animal disease and to develop novel and effective therapies. Finally, nowadays animals are increasingly used as therapeutic companions: as a matter of fact, there are evidences that they help ill people to recover faster, and help the aged live longer and more satisfying lives.

Nevertheless, the progressive changes of human lifestyles have induced modifications in the general attitude of humans towards the animals causing, in some cases, serious welfare problems. For example, in the pet-human relationships, often the status of 'family member' develops into behavioural problems. In the husbandry and management systems of farm animals, the passage from rural to more intensive and industrial production systems have strongly affected the quality of life of farm animals. In the scientific context, the use of sentient animals to study biological mechanisms of diseases, or to improve medical care, has been always a hot topic of debate in public and scientific community. In fact, nowadays, the enrolment of animals in scientific experiments is regulated by codes of practice and laws (see, for example, the Council Directive 86/609/EEC).

Why should we care for animal welfare? Mainly for two reasons: because to care for animal welfare it is advantageous for humans, and to because is a morally relevant activity.

Nevertheless, measuring animal welfare and evaluating different welfare issues can be very difficult. For example, the animals may fare better under conditions that seem less attractive to humans, than under those that we would instinctively prefer for them; we may misinterpret animals' responses and so wrongly judge their motivation and behaviour. Furthermore, it should be considered that many farm livestock and companion animals are quite different from their ancestors that faced a variety of threats from predators and disease, therefore "natural-ecological" solutions (perchery and free range systems for hens) not always may be the right choice. Therefore, we should also consider if the impact, on animal's well-being, of removing natural threats through domestication is more or less harmful to animal welfare than the stresses associated with intensive farming, or those suffered by animals housed in scientific laboratories.

The different topics presented in this volume have in recent years gained relevance both in theoretical and practical terms, recognised in an international scenario. Therefore, the choice to present the contributions of this volume in English will permit a wider diffusion of the ideas here presented.

BIOETHICAL CONSIDERATIONS ON THE USE OF GENETICALLY MODIFIED ANIMALS IN THE BIOMEDICAL RESEARCH

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In the last decades, the scientific community and the public opinion have became more and more aware about the issues concerning the use of genetically modified animals in the experimental and applied biomedical research. Such growing interest is highlighted by the considerable number of scientific and popular publications on this matter appeared in the recent years. Furthermore, since the potential risks relative to the use of the transgenesis techniques are not clearly predictable and the consequent ethical problems are not easy to be evaluated, this issue of the use of genetically modified animals is highly debated. Furthermore, international communities and commissions which have to monitor the effects of the development of new biotechnologies have some difficulty in being always updated to the last technique or methodology. Thus, sometimes they have a delay in the evaluation of the consequences of these biotechnologies for human health, for planet ecology and for animal welfare. Consequently, legislative and control organs are in a complex managerial situation.

For centuries, animals bearing specific combinations of genes in their genome were generated using traditional selection methods and without the current and relatively wide knowledge of genetics. This was a difficult approach since traditional selection methods have several limits. For instance, only conspecifics or individuals from species that are phylogenetically very close to each other can be bred. A further limit is represented by the fact that fate has a major role in determining the results obtained, and thus the time needed to produce the wished combination of genes was very long and involved a large number of generations. With respect to such approach, the transgenesis techniques consist in procedures radically innovative that allow to modify the genetic traits of interest very quickly, acting directly on the genetic material.

Rodents are the most commonly used species in research laboratories, because they have a very short inter-generation interval (around 2 months), need limited space for housing and require low maintenance costs. Among rodents, the mouse species (*Mus musculus*) has become the most used, both for the just mentioned reasons and especially because it offers an enormous advantage: a wide knowledge of its genome is available and the manipulation of its genome is easier compared to all other species. Indeed, in the mouse species, the transgenesis techniques have a relatively high probability to succeed.

Since the beginning of the Eighties, the use of genetically modified mice in the biomedical research has risen in exponential way, as it is shown by the increasing number of scientific publications on genetically modified animals available in the literature (Medline, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed). Indeed, the advantages brought by the use of the genetically modified animals to the scientific research are clear and important (Crawley, 2000). Among the most relevant are: (i) specificity, the new techniques allow to specifically target the gene of interest, abolishing or at least limiting the role of fate; (ii) rapidity, a specified combination of genes can be obtained in only one generation; (iii) flexibility, since the DNA, the deoxyribonucleic acid, is structured according to rules of a universal code common to every living organism, it is possible to transfer genetic material and

thus information between species also phylogenetically very distant from each other, and (iv) the low cost, the techniques can be carried out on animal species, as in the case of the mouse, whose maintenance costs are limited, allowing to work on a relatively large number of individuals.

On the contrary, the problems concerning the development and use of the transgenesis techniques, are much less easily estimable and predictable, and principally concern two issues: i) ethical issue, the modification of the genome can, directly or indirectly, cause pain to the experimental animals; ii) practical issues, genetically modified mice could escape from the animal facilities where they are housed and may breed with the endemic populations, leading potential serious ecological perturbations.

The issue of animal welfare, when genetically modified animals are used, should be considered taking into account the specific genetic and consequently phenotypic alterations of the experimental subjects under examination (Hazecamp & De Cock Buning, 1998; Duncan & Fraser, 1997; Vitale & Alleva, 1999; De Cock Buning, 1999). The generation of transgenic and-out knock mice may cause unexpected side-effects that could have heavy consequences on animal welfare, like a high pre-natal or neonatal mortality or an abnormally increased or decreased fetal weight. However, the alterations induced by the genetic manipulation may lead also to less subtle and less evident effects such as a modification of the response to stress or of the pain threshold. For instance, iper- or ipo-algesia (respectively increased or reduced sensitivity to pain), motor or sensory weakness, low social interaction capacities, etc., can make the animal especially susceptible to stressful conditions. These effects should be always monitored, especially in studies aimed at characterizing the behavioral response.

Ethical guidelines regulating the use of genetically modified mice should be aimed at improving the current research strategies by exploiting "3Rs" model and innovative statistical methods (Russell & Burch, 1959; Ghislaine *et al*., 2000). The "3Rs" model was created in 1959 by two English researchers, Russell and Burch, and it still represents a key reference point for the establishment of new experimental protocols that take into consideration rights and needs of laboratory animals. Briefly, the model is based on three main principles: (i) Reducing the number of the animals involved in experimental protocols; (ii) Refining experimental procedures in order to improve the quality of life of experimental subjects; and (iii) Replacing laboratory animals with *in vitro* models and computer simulators or, at least, with other animal species characterized by a less complex nervous system and thus having a relatively lower vulnerability to pain (Ekwall *et al*., 1990).

With regard to the production of genetically modified animals, the principle of Reducing is hard to be addressed. Indeed, a rapidly increasing amount of individuals, especially mice, is used for transgenesis experiments. In this framework, a progressive refinement of statistical methods can contribute to the reduction of the number of used animals, without reducing the power of behavioral analyses and without compromising the quality of the results obtained (Chiarotti & Puopolo, 2000; Trajstman, 2000). With regard of the Refining principle, an ethological approach is warranted to improve housing and experimental conditions of laboratory animals (Vitale & Alleva, 1999; Alleva & Carere, 2000). This approach brings advantages at various levels: on the one hand, treating experimental subjects gently and avoiding exposing them to stressful procedures abolish, or at least reduce, animal sufferance; on the other, a direct consequence of the enhanced psychophysical welfare of experimental subjects is a more reliable behavioral response pattern, improving data quality.

Though the mouse is the most massively used species in the biomedical research, attention should be paid also to all other species increasingly used for transgenesis experiments. This is particularly true because the development of the techniques now makes easier to genetically modify also other species, such as rats, sheep or swine.

The use of genetically modified animals presents several practical pitfalls. In order to avoid these, a number of precautions should be followed. Special attention should be paid to accurately control the environment in which the modified gene is expressed in order to control for possible pleiotropic effects: same gene modifications produces markedly different results when expressed in different mouse strains, and thus in different genetic backgrounds. The time of expression of the gene of interest should be monitored to avoid misinterpretations. For instance, changes rising as a consequence of the absence of the gene of interest at early ontogenic phase may heavily interfere with the normal developmental program, leading to physiological and/or behavioral modification that can be erroneously interpreted as consequences of the lack of the gene at adulthood (Newmark, 1989; Lipp & Wolfer, 1998). Moreover, there are other factors that can confuse the interpretation of the phenotype, such as the insertion of genes adjacent to the one of interest, the so-called "flanking" genes, which can introduce unexpected variability hard to control. Special attention should be paid to the influence of the mouse strains used to produce the genetically modified lines. For instance, the C57B6 mouse strain, which is widely used for transgenic and knockout production, is characterized by congenital deafness that makes these mice absolutely not suitable when hearing is a key sensory modality to accomplish the test administered by the experimenter. Another strain, the FVB mouse, undergoes to retinal degeneration and therefore these animals may show important impairment in visual tests such as the Morris water maze. Other strains may lack of specific molecules and consequently may show different phenotypic traits. Finally, as previously mentioned, pleiotropia, the phenomenon for which a single gene affects several characters of the same organism, is very common and makes extremely difficult to identify the biological effects even of a single gene mutation (for further details on this matter please see Gingrich & Hen, 2000; Gerlai, 1996).

When working with genetically modified animals, the risk of contamination of the wild ecosystems must not be underestimated. Transgenic or knockout mice escaped from animal facilities, especially if the genetic mutation is favored by the natural selection, may go in contact with the endemic mouse populations living in the natural environments surrounding the research laboratories. This may represent a danger for the wild population that may be contaminated or, in the worst case, may be forced to extinction. Unfortunately, there are many examples in which the introduction of new mammal species can cause great danger to the endemic populations (Baskin, 1996; Baskin, 1998). The prevention of the escape of experimental subjects must be therefore a priority for the all researchers working in facilities containing genetically modified animals.

In conclusion, it is urgent to develop or update strategies aimed at controlling the effects of innovative and promising biotechnologies in order to guarantee not only human health but also the welfare of experimental animals. Finally, the public opinion may consider the production of genetic modified mouse as a challenge to the animal's intrinsic value (an important Italian economist talked once about alteration of the structure of the living matter). Though scientist may disagree on this point, such reaction leads to a crucial moral theme and should be neither neglected nor considered as not important by scientists. Indeed, though the advantages offered by transgenesis techniques are clear and indubitable, the use of genetically modified animals raises new ethical and practical problems that require original solutions.

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APPLICATION OF THE "3RS" MODEL IN BIOMEDICAL EXPERIMENTATION: THE CASE OF THE STUDY OF THE NEUROBEHAVIOURAL TOXICITY EFFECTS AFTER DEVELOPMENTAL EXPOSURE TO ANTIRETROVIRAL AGENTS

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The "3Rs" model

In 1959 two British academics, Rex Burch and William Russell, members of the University Federation of Animal Welfare (UFAW), published a book destined to become a fundamental and lasting reference for animal welfare science. In their book the two authors proposed a methodological recipe, aimed at helping the researcher to perform experiments on animals in a more "humane" way. This recipe is called the "3Rs" model, or the rule of the "3Rs".

The "3Rs" stands for: Replacement, Reduction and Refinement.

Replacement

Firstly, the researcher should try as much as possible to replace the animal with an alternative method. In the definition by Russell and Burch, Replacement is described as: "any scientific method employing non-sentient material which may, in the history of experimentation, replace methods which use conscious living vertebrates" (1).

Therefore, the notion of Replacement originally offered by the authors was referring to the substitution of an animal experiment by an experiment, method or procedure which used exclusively non-sentient material. However, Russell and Burch made a distinction between complete Replacement and relative Replacement. With relative Replacement, in some phases of the experimental procedure, the use of animals is still necessary, for instance because animals are killed for organs to derive cells for *in vitro* cultivation. The up-dated interpretation of Replacement includes also the possibility to choose an animal with "less complex" nervous system from the one originally presented: for example, a mouse for a monkey; an invertebrate for a vertebrate. The underlying assumption is that the less sophisticated the neurological development, the less the amount of potential suffering caused by experimental procedures. This notion is also encoded in the current European legislation on the protection of animals used in experiments (2). However, this assumption is in some way debatable: a large range of animals, which includes invertebrates, experiences "pain". For example, there are some neurochemistry overlapping between vertebrate and invertebrates, for what concerns pain reception and mediation (3, 4).

If we want to intend the term Replacement in a broader sense, we could imagine different options: i) we could think to replace some technique which is part of the experiment; ii) to replace the kind of protocol to answer a specific question; iii) to replace a scientific or policy plan: for example, abolishing animal experiments in the production and testing of cosmetics.

(5). These examples show how far can the "3Rs" model be applied, and how broad are its potential theoretical and methodological implications.

Reduction

When Replacement is not possible, the researcher should try as much as possible to Reduce the number of individuals utilised in a certain experimental protocol. Reduction strategies can be applied in situations where animal models are used, aiming at the smallest number of animals that still leads to a scientifically valid or worthwhile result. This aim can be fulfilled using appropriate statistical methods, which can suggest, for example, the minimum number of individuals needed in a particular experimental design in order to apply a particular statistical test.

A broader way to apply the concept of Reduction could be a better communication between different laboratories, in order to avoid useless replication of similar experiments, therefore reducing the total number of animals utilised. Therefore, as in the case with Replacement, Reduction can refer to scientific procedure in a wider sense, that is, within a certain experimental protocol, between different experimental protocols, between different scientific areas of research.

Refinement

In theory, Refinement starts when we cannot use Replacement techniques, and every device of theory and practice has been employed to reduce to a minimum the number of animals used in experiments. The aim is to refine experimental procedures to minimise, alleviate or eliminate pain and sufferance inflicted upon experimental animals.

Russell and Burch indicated Refinement as "any decrease in the incidence or severity of inhumane procedures applied to those animals which still are to be used" (1). One of the results of a theoretical study recently carried out by a multidisciplinary group (www.inemm.cnr.it/animalsee.html), involving biologists as well as philosophers, was the re-definition of this concept as follows: "Any approach which avoids, alleviates or minimises the actual or potential pain, distress and other adverse effects suffered at any time during the life of the animals involved, or which enhances their well-being as far as possible" (6). This definition includes all aspects of animal's life in which refinement techniques can be applied: housing and husbandry, techniques used in scientific procedures, procedural care and experimental design. It also calls for an active role by the researcher in trying to ameliorate the captive conditions of the experimental subjects.

The relation between Reduction, Replacement and Refinement

The "3Rs" of Russell and Burch's model can interact one with each other. As a matter of fact, Replacement can lead to the overall Reduction in animal experimentation. Refinement techniques can have an impact on the number of animals used, then interacting with Reduction: for example, the use of humane endpoints or the use of positive training. Furthermore, less stressed animals could reduce variability within an experimental sample, then decreasing the need for duplication of research.

In other situation, the "3Rs" can be in conflict with each other: For example, in Parkinson's disease studies it is possible to reduce response variability to the treatment performing intracarotid injection of MPTP, rather than intra-muscular. This method has been successfully

used in macaques, obtaining at least 70% rate of success in producing unilateral parkinsonism, with a near-to-zero mortality. Furthermore, these subjects remained parkisonian for years (7). However, in this case we can see a possible conflict between Reduction and Refinement. On the one hand, the possibility to work on the same subjects for several years decreases the number of individuals to be injected for the completion of the study; on the other, the amount of suffering caused by the long illness induced on these subjects mean a prolonged and significant decrease in their level of welfare, somehow against the concept of humane end-point (8). Genetic modification of experimental animals may have the potential to reduce the number of animals, but some studies have shown serious adverse effects of genetic modification on animals' health and welfare.

The "3Rs" model and the use of rodents

Preclinical studies involving animal models are currently used in the attempt to predict the likelihood that an agent will produce adverse effects in humans. Complex risk assessment protocols are defined by regulatory activity to protect human population from exposure to toxic agents, they are either environmental or pharmacological agents.

During the last four decades neurobehavioural toxicology has increased its influence on regulatory activity by both European and United States governmental agencies in considering that functional assessment may have greater sensitivity the histopathological and biochemical methods. Several expert panels have recommended in toxicity hazard identification the use of neurobehavioural endpoints, such as motor and sensory functions, cognitive skills, social competencies, often in conjunction with other measures of neurotoxicity such as neuropathology and neurochemistry. In parallel, in particular after the thalidomide tragedy, behavioural teratology research disclosed the importance of safety evaluation and developmental neurobehavioral toxicology testing become one of the regulatory requirement in the assessment of the consequences of therapeutical or chemical agent exposure during pregnancy or earlier postnatally. As a matter of fact the neurotoxic effects produced by the exposure during development to chemicals or drugs may be rather different from the consequences inflicted on a mature brain. Thus preclinical studies focused on the analysis of different dose and timing of exposure and aimed to track long-term effects of developmental insults may increase knowledge about the toxicity hazard both in child and adult humans.

The animal studies on the developmental neurotoxicity effect of antiretroviral drugs could be considered a paradigmatic example of risk assessement by neurobehavioural testing. Thus consideration about the applicability of "3Rs" model to such a study could improve the experimental approach towards neurotoxicity risk evaluation by preclinical studies.

During the past 10 years effective reduction in maternal-fetal HIV-1 virus transmission has been achieved by administration of zidovudine (AZT) to HIV-infected women, and their newborns during pregnancy and early neonatal period. In spite of such benefits, there is still considerable uncertainty on the potential long-term adverse effects of antiretroviral agents on exposed children. AZT is an antiretroviral agent belonging to the class of nucleoside analogues (dideoxynucleotides) acting as inhibitor of viral reverse transcriptase Furthermore, *in vitro* data (9) demonstrate that nucleoside analogues such as AZT interact with β-polymerase (nuclear enzyme implicate in DNA repair) and significantly inhibits γ-polymerase (enzyme implicate in mitochondrial DNA replication). Thus, treatment with antiretroviral agents could lead to respiratory chain disorders at mitochondrial level as well as accumulation of mutations in the genomic DNA with potential oncogenenic effect.

There is still equivocal evidence regarding *in utero* NRTI exposure and mitochondrial dysfunction in HIV-uninfected children born of HIV-infected women. Symptoms compatible with mitochondrial dysfunction have been reported in HIV-uninfected children exposed to NRTIs, and AZT-DNA incorporation in leukocyte genome as well as mtDNA depletion has been evidenced in cord blood from NRTI-exposed children in comparison to non-exposed children. Evidences pointing to mitochondrial dysfunction in NRTI exposed children indicate a prevalence of neurological signs. Emotional and behavioral abnormalities, including social problems, anxiety, and depression have been reported in a large proportion of children with perinatal acquired HIV infection. It is worth noting that children and adolescents born to HIV-positive mothers but uninfected by the virus still present significant behavioral problems. Thus NRTIs might constitute a comorbid risk factor contributing to such neurodevelopmental abnormalities. A very recent report based on a large cohort of uninfected children exposed to NRTIs in utero concluded that, while there was no significant association between overall *in utero* NRTI exposure and mitochondrial dysfunction, data concerning NRTI exposure in the third trimester of pregnancy were still not conclusive.

Studies on animal models support the view that NRTI exposure per se induces long-term effects on behaviour that are consistent with the disorders reported in NRTI-exposed children. The behavioral endpoints affected by perinatal exposure to AZT or AZT+3TC combination include sensorimotor maturation, social/aggressive behavior, activity levels, while learning abilities are relatively spared. The behavioral changes observed in the mouse species at the juvenile and adult stages suggest that the neural systems modulating emotional/anxiety states are the main targets of NRTIs.

Based on these observations, the use of rodent models could provide a comprehensive assessment of the developmental neurobehavioral toxicity of antiretrovirals with potential transferability to humans by the analysis of behavioural endpoints paralleled with analysis of brain damage in term of neurochemical and neuropathological markers.

Replacement

The Replacement principle asks the researcher to evaluate the use of alternatives to the use of animal models, or the use of animals characterised by a less complex nervous system.

Data collected so far in animal experiments employing laboratory rodents such as rats and mice have provided important indications as to the mechanisms by which different xenobiotics may influence brain and behaviour development in humans.

Rodents are successfully used in the study of potential behavioural changes due to exposure to noxious agents during development. Although there are important differences between the rodent and human brain, analogous structures can be identified that subserve behaviours with close functional value. In general, regional development of the rodent brain proceeds on a timeline of days versus weeks to months in humans, although gross development of the brains of rodents and human is similar (10). A newborn rat/mouse is comparable in stage of brain development to the human foetus at the end of the second trimester of gestation and a 6 to 10-day-old rat/mouse is comparable to an infant at term (11). The behavioural ontogeny of laboratory mice has been widely characterized, and several validated experimental paradigms are available to reveal even subtle alterations induced by neurotoxic agents. Different behavioural endpoints related to sensory, motor, motivational, cognitive and social functions can be measured, taking into account both age and sex specificity. Furthermore rodents rapid maturation (they reach puberty around postnatal day (PND) 35 and sexual maturation at 60 days) allows one for an extended follow-up of treatment effects in a relative short time span.

Though any extrapolation from rodents to humans must be carefully considered, a large body of evidence [see data on developmental exposure to lead and metylmercury (12) alcohol (13), pesticides (14)] has indicated that for many neurotoxicants the behavioural abnormalities observed clinically are mirrored in rodent models (15, 16). However, comparative studies on rodent and human data show that there are some gaps in the face validity of the rodent behaviour that are difficult to fill up: for example, many behavioural tasks which involve visual and language competencies are not modelled in rodent models. Furthermore, though analogous neurobehavioural targets are involved in rodents and humans for the greater number of compounds there are some limits in the current risk assessment using neurobehavioural data especially for quantitative aspects of hazard evaluation. Indeed, behavioural data are often characterised by phenomena such as reversibility of the effects, different susceptible populations, and not linear dose-effects curve (17).

Therefore, rodent models in neurotoxicology are still the preferred option. Nevertheless, we can here cite some possible alternatives.

The in vitro methods are increasingly developed in toxicological study, but they can mainly help to understand specific mechanisms involved at tissue, cellular and molecular level. Nevertheless, they appear completely insufficient – and barely predictive – of alterations of integrate functions such as the behaviour. In vivo studies are commonly utilised in basic research to assess the effects of exposure of a test substance during development and to evaluate potential hazard to human for chemical and pharmaceutical regulation. Studies with animals permit intensive and methodological evaluation of important parameters, such as dose-response relationships, critical periods of susceptibility, and the relative contribution of genetic and environmental factors.

Data collected so far in animal experiments employing laboratory rodents such as rats and mice have provided important indications as to the mechanisms by which different xenobiotics may influence brain and behaviour development in humans.

In terms of partial Replacement, the zebrafish (Danio rerio) is largely used in toxicological studies and in particular in those focused on toxic effects on development. There are several advantages to use zebrafish model in toxicological screening: the size (3.5 cm), the high fecundity level (200-300 eggs in a morning, laid every 5-7 days) and the transparency of the eggs, which allows direct observation of organ and tissue malformations also with the aid of fluorescent markers (18). Furthermore this fish can live in as little as 50 ml of fluid, thus only micrograms of compound are needed for screening per assay. This is facilitated by the fact that zebrafish readily absorbs compounds from the water (198). There is a growing literature on the use of zebrafish to model human diseases and such a model has been introduced in behavioural teratology study for the assessment of neurotoxicity of compounds such as pesticide, pollutants or endocrine disruptors. Data collected in these studies demonstrated that pesticides could modify both motor and cognitive function of this species (20). However, the role of this model, while well corroborated in the study of disease processes, appears weakly in term of face validity when toxicological study foresees to collect data, which are informative on specific mammal specialisations such as complex social behaviour, dyadic mother-to-pup relationship, and emotional component of cognitive capability.

The Japanese quail (*Coturnix japonica*) has been used in several studies aimed to analyse the potential dangerous effect of chemicals on sexual behaviour and reproductive performance, overall defined as endocrine disruptors (21, 22). The advantage to study such effects in birds is due to high rates of food consumption, high metabolic rates, periods of starvation that mobilize lipid reserves, hormone-dependent behaviours, developmental strategies, and control of sexual differentiation. Ultimately, the biological fitness of a bird can be dramatically affected by very subtle changes in the normal functioning and balance of its endocrine system. Avian models

thus offer the opportunity to have a multilevel approach in studying the neuroendocrine developmental toxicity of a compound. Tests that solely examine the effects of potential endocrine disruptors on the morphology and functioning of organ systems may not be adequate to determine the complete toxicity of a compound. Intact and functional gonads do not contribute to an individual's fitness if the performance of reproductive behaviour is impaired. The strict dependence of bird behaviour, developmental strategies and sexual differentiation on hormones provides a method of linking physiological function with eco/ethological processes. Although avian models have largely contributed to clarify the developmental and neuroendocrine targets of several potential dangerous compounds, their use in laboratory study is limited by some difficulties in the husbandry conditions of this species, and, further on basis of the poor number of comparative studies aimed to evaluate the face validity of this model towards human health concerns.

Reduction

The use of improved experimental design and statistical analysis techniques are key means of achieving the Reduction of the experimental subjects utilised in a particular experimental protocol. Experiments should be planned before they are started, and this planning should include the statistical methods used to assess the results.

Behavioural teratology studies need a large number of animals to be tested as they compare more dose-treatment groups, investigate on difference in sex, age and genetic susceptibility, and often are interested in collecting biochemical data which can be correlated to behavioural effects potentially made known.

Table 1 illustrates the number of animals predicted to be used in two experiments: the first aiming at investigating on NRTI developmental exposure on specific neonatal behavioural and biochemical endpoints and the second to analyse behavioural and biochemical effects at adolescence and adulthood. The total number predicted is 810, that is, a considerable amount of animal subjects. However, this number can be justified by the particular design of the experiment.

Table 1. Number of animals forseen in the experimental design

First year	Mother		Offspring		total
			males	*females*	
Behavioural effects in neonatal period (experiment 1)	15	vehicle	60	60	
	15	NRTI	60	60	
total	30		120	120	270
Behavioural effects at adolescence and adulthood (experiment 2)	15	vehicle	60	60	
	15	NRTI	60	60	
total	30		120	120	270

As illustrated in Figure 1 the experiment is planned following a split-litter design. From each mother administered with NRTI during gestation we obtain four pups in each sex (this is a conventional number to which many laboratories reduce the number of the rodent offspring).

Each one will be assigned to a different test. Thus through the drug administration to one subject (the mother), a magnification of the sample exposed to NRTI will be obtained, with a significant optimization of the experimental design in terms of: the number of animal used, drug amount needed, facilities cost, and last but not least hours of work. As an example for each 45 administered females group (see Figure 1), the split-litter design used in this study allows to collect i) both behavioural and neurochemical data; and ii) information on interaction between drug exposure and sex (the same test was replicated in each sex).
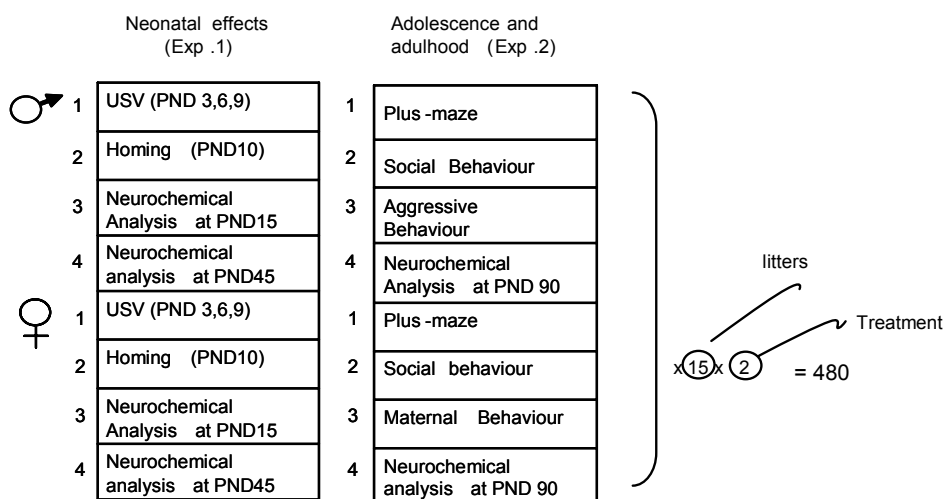
Figure 1. Animals assignment to neurochemical and behavioural analysis

This study utilises an animal model of development, where treatment is randomly administered to mouse females during pregnancy, thus pup exposure will be obtained through the mother. Because of planning, the experiment in this way the statistic unit to consider in the statistical analysis of data will be the litter [for more detail see (23)]. To better clarify, in this experiment quantitative behavioural and biochemical responses will be analysed in terms of the following fixed factors: the treatment factor (two levels: NRTI and vehicle), the sex factor (two levels: male and female), and, in the case of ultrasound vocalisations (UVZ) and aggressive behaviour, a repeated measure factor (three levels, corresponding to the three repeated days of testing). Furthermore, each of these factors is in a specific relation with the statistical unit litter. Treatment is a between-litter factor and litters are nested within treatment: different treatments are administered to different litters (mothers and through them, to their offspring). On the contrary, sex and day of testing are within-litter factors: from each litter one male and one female (which are littermates) are analysed and each pup of each sex from each litter will be tested at several days (see Fig. 2). In this kind of studies data should be collected according to a factorial design and analysed by a parametric analysis of variance (ANOVA), to assess the significance of the main effects of each investigated factor and their interactions. In particular, a split-plot ANOVA with litters nested under treatment and blocking factor for sex and day of testing should be applied.

The decision on how many animals to use in an experiment is of crucial importance for both ethical and scientific reasons. An experiment that is too small may miss biologically

important effects, whereas an experiment that is too large wastes animals. A power analysis is the most common way of determining sample size. Formulae are available to compute the appropriate sample size based on the following parameters: the (1) effect size of interest, (2) standard deviation (for variables with a quantitative effect), (3) chosen significance level, (4) chosen power. Although the formulae are complex, particularly for more advanced designs, it is usually possible to simplify the questions so that a good approximation of the optimum sample size can be obtained using one of the available statistical packages or an interactive web site (24).

In the study mentioned above sample size estimation was carried out on a specific behavioural response namely ultrasound vocalizations, since such endpoint is more variable and therefore more critical from a statistical/sample-size point of view. Due to the strong variability and asymmetry (longer upper tail) of USV distribution and proportionality between group means and standard deviations, USV data are usually transformed using the square root transformation. Therefore, the minimum required sample size was computed considering the two-tailed Student t test for independent groups using the following values, based on the results of previous studies. Specifically, the following figures were taken into consideration: 1) effect size of interest, minimum difference between control and treatment group means that we would like to find out as significant Δ = 7.2 (i.e. $\Delta = 1.30*\sigma$, corresponding to the 80% reference interval of control subjects); 2) standard deviation homogeneous among groups $\sigma = 5.5$; 3) chosen significant level, Type I error probability $\alpha = 0.05$ (conventional value); 4) power $1-\beta = 0.80$ (conventional value). The sample size resulting from this calculation was 10 subjects per treatment group. As can be noted the sample size reported in Figure 1 is 15 subjects for each treatment group. This number was calculated taking into account the power analysis applied to USV variable, but it has been incremented because the potential loss of subject due to experimental procedures such as failure of pregnancy, poor reproductive performance (less pups than expected), individual vulnerability to route of administration and/or drug.

Overall, the number of animals foreseen in this study is very elevated. However, the presence of a well designed experimental plan as well as the accuracy in the choice of statistical methods guarantee a large number of information to be collected under the control of statistical power and reliability of data.

Refinement

Husbandry system and experimental procedures mainly respond to hygienic, economic/ergonomic as well as standardisation requirement and usually have poor consideration for animal welfare. However, in the recent years growing consideration for the welfare has encouraged new fields of research aimed to improve comfort and limit sufferance of laboratory animals. Several studies indeed demonstrate that through Refinement techniques, such as environmental enrichment, an amelioration of the condition housing and maintenement of animals can be achieved. At the same time, Refinement of experimental procedures can significantly reduce the level of sufferance of experimental animals. However, the use of refinement techniques should be informed by eco/ethological considerations related to the species and/or strain used as experimental subjects. Physical and social stimuli can act both as stressors and positive drive in respect to their 'natural' value, thus research of the actual meaning of enrichment strategies to use in laboratory [beneficial enrichment *versus* stimulation, see (23)] should be promoted to evaluate consequences on both welfare and data quality.

There are at least three critical points in the experimental design proposed in the case

study here analysed that needs to be addressed properly to limit possible suffering in experimental subjects:

i) Route of administration: the drug under study is administered *per os,* intragastrically, using a 3-cm long curved feeding needle attached to a 1-cm^3 syringe. This kind of treatment involves a potential disruptive manipulation specially considering that treatment is made on females in the third trimester of pregnancy. Many rodent studies show that the stress imposed during pregnancy results in alterations of mother and offspring behaviour. These effects can therefore introduce a bias in a study in which analysis of behaviour is the main scientific objective. Therefore, the implementation of alternative route of administration could diminish sufferance of animals and improved quality of behavioural data collected.

ii) Use of neonate animals: it has to be taken into account that the newborn's behaviour and physiology are regulated by olfactory, thermal, and tactile stimulation supplied by the mother in the nest environment. Thus, any disturbance to the mother- offspring relationship in the first two weeks of life can induce in the neonate rodents a change in their physiological and psychological state. Because this study intends to investigate the response of neonate mice exposed developmentally to NRTIs to separation from familiar cues from the nest, attention should be paid to reduce the duration of separation from the mother to the minimal amount of time possible (3 min are necessary for recording of the ultrasound vocalizations emitted in isolation), and, to keep thermal condition of isolated pup near to the physiological level.

iii) Aggressive interactions between males: Careful monitoring of the agonistic encounters between males is the rule in advanced laboratories, where personnel has specific expertise in scoring social/agonistic repertoire in rodents. Whenever a member of the fighting pair displays high aggression, reflected in repeated biting, the encounter is interrupted to avoid serious injury. Finally, the experimental subjects should be housed as to guarantee a satisfactory level of welfare in relation to physical and social needs typical of the species.

Conclusion

In conclusion, the examination of the neurobehavioural toxicity study on the effects of developmental exposure to antiretroviral agents by means of the replacement, reduction and refinement requirements shows that the "3Rs" model is a powerful tool in order to evidence critical point in the ideation and planning of experimental study, which involve the use of animal models. As for the use of rodents as experimental model, this choice appears appropriate for the purpose of the study, which is aimed to investigate detrimental effects on complex behaviour patterns that are well characterized in these species, and for which several validated experimental paradigms are available to reveal even subtle alterations induced by neurotoxic agents. Some limits emerge in the attainment of reduction requirement, especially due to the strong variability of behavioural measures and to the need to collect information such as age and sex differences in susceptibility to antiretroviral agent. Thus, the use of advanced statistical methods appears essential in this kind of study in order to reduce as more as possible the number of animals involved. Refinement procedures, especially in studies which involved behavioural measures strongly influenced by health condition and environmental factors, are likely the level of intervention on which researchers may improve their experimental plans. Respect of eco/ethological characteristics of the species in use should increase in the organization of husbandry system as well in the development of experimental procedures.

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LABORATORY ANIMAL WELFARE IN EUROPE AND NORTH AMERICA

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Introduction

Together with the development of the use of laboratory animals for research purposes in Europe, criticism against animal experimentation began to grow in England. The public debate about the use of laboratory animals brought to the creation in 1875 of the Victorian Society, the first antivivisectionist organisation. The following year the first legislation, aimed at protecting the welfare of animals used for scientific purposes, the "Cruelty to Animal Act", was adopted by the British Parliament. According to the provisions of the law, researchers were required to register the number of animals, and to specify as well the scientific purposes for which these animals were to be used. The continuous development and the increase in the number of animals used in research prompted in the following century the adoption of national legislation in different countries together with international declarations and conventions.

Declaration of Helsinki

The Declaration of Helsinki was adopted by the World Medical Association General Assembly in June 1964; although its principal aim is to state the ethical principals to be followed when medical research involving human subjects is carried out, this declaration in its basic principles recognises the importance to use laboratory animals and to guarantee their welfare. In fact its article 11 reads:

> Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation", and its article 12 reads "Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Universal Declaration of Animal Rights

Another important text, which has been adopted to protect animals, is the Universal Declaration of Animal Rights. It was solemnly proclaimed on the 15th of October 1978 at the UNESCO (United Nation Educational Scientific an Cultural Organisation) headquarter in Paris, it was revised by the International League of Animal rights in 1989, and in 1990 was submitted to the UNESCO Director General and made public. The text of the declaration is the following:

Preamble:
- Considering that Life is one, all living beings having a common origin and having diversified in the course of the evolution of the species, - Considering that all living beings possess natural rights, and that any animal with a nervous system has specific rights, - Considering that the contempt for, and even the simple ignorance of, these natural rights, cause serious damage to Nature and lead men to commit crimes against animals, - Considering that the coexistence of species implies a recognition by the human species of the right of other animal species to live,- Considering that the respect of animals by humans is inseparable from the respect of men for each other,

It is hereby proclaimed that:

Article 1
All animals have equal rights to exist within the context of biological equilibrium. This equality of rights does not overshadow the diversity of species and of individuals.

Article 2
All animal life has the right to be respected.

Article 3
1°- Animals must not be subjected to bad treatments or to cruel acts.
2°- If it is necessary to kill an animal, it must be instantaneous, painless and cause no apprehension.
3°- A dead animal must be treated with decency.

Article 4
1°- Wild animals have the right to live and to reproduce in freedom in their own natural environment. 2°- The prolonged deprivation of the freedom of wild animals, hunting and fishing practised as a pastime, as well as any use of wild animals for reasons that are not vital, are contrary to this fundamental right.

Article 5
1°- Any animal which is dependent on man has the right to proper sustenance and care.
2°- It must under no circumstances be abandoned or killed unjustifiably.
3°- All forms of breeding and uses of the animal must respect the physiology and behaviour specific to the species.
4°- Exhibitions, shows and films involving animals must also respect their dignity and must not include any violence whatsoever.

Article 6
1°- Experiments on animals entailing physical or psychological suffering violate the rights of animals.
2°-Replacement methods must be developed and systematically implemented.

Article 7
Any act unnecessary involving the death of an animal, and any decision leading to such an act, constitute a crime against life.

Article 8
1°- Any act compromising the survival of a wild species and any decision leading to such an act are tantamount to genocide, that is to say, a crime against the species.
2°- The massacre of wild animals, and the pollution and destruction of biotopes are acts of genocide

Article 9
1°- The specific legal status of animals and their rights must be recognised by law.
2°- The protection and safety of animals must be represented at the level of Governmental organizations.

Article 10
Educational and schooling authorities must ensure that citizens learn from childhood to observe, understand and respect animals.

By reading its text, it appears clear that the spirit of the universal Declaration of Animal Rights is based on the principle that all the species are equal with regard to life; as far as experimental animals are concerned, it both underlines the importance of developing alternative methods, and affirms that any suffering caused to animals for scientific purposes is a violation of their rights.

Legislation in North America

Canada

While Canadian federal legislation covers the prevention of cruelty to animals through the Criminal Code, it does not include experimental animals if their use can be demonstrated to be necessary; in some provinces, such as Alberta, Ontario, Saskatchewan and Quebec, specific pieces of legislation on laboratory animal welfare have been implemented. The Canadian Council of Animal Care (CCAC), which is a national, peer review organisation, was founded in 1968 in Ottawa under the aegis of the Association of Universities and Colleges of Canada and it became a non-profit independent, and autonomous body in 1982. The mandate of CCAC states:

> The purpose of the Canadian Council on Animal Care is to act on behalf of the people of Canada to ensure, through programs of education, assessment and persuasion that the use of animals in Canada, where necessary for research, teaching and testing employs physical and psychological care according to acceptable scientific standards, and to promote an increased level of knowledge, awareness and sensitivity to the relevant ethical principles.

The CCAC comprises 22 Member Association, whose representatives include scientists, veterinarians, educators, and delegates from industries and animal right movements; it mainly obtains its public funding through the Natural Sciences and Engineering Council of Canada and the Medical Research Council of Canada, with the additional contribution from some private institutions

It formulates guidelines, which are aimed at guaranteeing animal welfare and at avoiding unnecessary animal suffering; it organises assessment visits to research institutions to verify their compliance with its guidelines, which is a requirement for the receipt of grants.

United States

In the United States Congress has approved a set of laws whose enforcement has been delegated to different agency.

The principal laws concerning the protection and welfare of experimental animals are listed below.

Animal Welfare Act was passed in 1966 an amended in 1970, 1976, 1985, and 1990; the Animal Welfare Act is enforced by the United States Department of Agriculture, and it sets minimum standards of treatment and care animals should be provided with, when they are bred for commercial sale, used for research, or exhibited to the public. The species the Animal welfare Act applies to are specified in its definition of animal, which reads

> Animal means any live or dead dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or any other warmblooded animal, which is being used, or is intended for use for research, teaching, testing, experimentation, or exhibition purposes, or as a pet. This term excludes:

Birds, rats of the genus Rattus and mice of the genus Mus bred for use in research, and horses not used for research purposes and other farm animals, such as, but not limited to livestock or poultry, used or intended for use as food or fiber, or livestock or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber. With respect to a dog, the term means all dogs, including those used for hunting, security, or breeding purposes.

It is worthy noticing that only rodent of the genera *Mus* and *Rattus* are excluded, while other rodents less used in medical research, such as hamsters, are covered by this act. This exclusion has prompted an ongoing public debate, and American Animal Right Organisations have made several attempts to change the definition of animal in the act. They argue that mice and rats make up 95% of animals used for research purposes, and that their inclusion in the Animal Welfare Act is essential and humane; on the other hand research groups contend that they are already covered by other regulations and that their inclusion in the Animal Welfare Act would increase routine cost of animal maintenance without improving their welfare.

The Public Health Service Policy on Humane Care and Use of Laboratory Animals was published in 1986 to incorporate the changes required by the Health Research Extension Act (Public Law 99-158, 1985); it defines as animal:

Any live, vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes" and it applies to "all PHS-conducted or supported activities involving animals, whether the activities are performed at a PHS agency, an awardee institution, or any other institution and conducted in the United States, the Commonwealth of Puerto Rico, or any territory or possession of the United States. Institutions in foreign countries receiving PHS support for activities involving animals shall comply with this Policy, or provide evidence to the PHS that acceptable standards for the humane care and use of the animals in PHS-conducted or supported activities will be met.

This policy includes both intramural and extramural NIH (National Institute of Health) research. It is interesting to notice that the need to comply with the above mentioned regulations changes according to the animal species used or the source of funding: e.g an Institution, which is internally funded and which uses only mice and rats, is neither subject to the Animal Welfare Act, nor to the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Legislation in Europe

The protection of animals, used for research purposes, is regulated in Europe by the specific legislation of each European country and by supranational regulations which have been adopted by two different organisations, The Council of Europe and the European Union.

European Supranational regulations

The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes

The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes was adopted by the Council of Europe adopted on the 18th of March 1996, and entered into force the 1st of January 1991. It has been signed by Belgium, Bulgaria Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland,

Lithuania, Netherlands, Norway, Portugal, Romania, Slovenia, Spain, Sweden, Switzerland, Macedonia, Turkey, United Kingdom and the European Union. It has the been ratified Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Netherlands, Norway, Portugal, Romania, Slovenia, Spain, Sweden, Switzerland, Macedonia, United Kingdom and the European Union.

The Convention is composed of 37 articles and two appendices: Appendix A "Guidelines for accommodation and care of animals" and appendix B "Statistical Table" and its preamble reads:

> "The member States of the Council of Europe, signatory hereto,
> Recalling that the aim of the Council of Europe is to achieve a greater unity between its members and that it wishes to co-operate with other States in the protection of live animals used for experimental and other scientific purposes;
> Recognising that man has a moral obligation to respect all animals and to have due consideration for their capacity for suffering and memory;
> Accepting nevertheless that man in his quest for knowledge, health and safety has a need to use animals where there is a reasonable expectation that the result will be to extend knowledge or be to the overall benefit of man or animal, just as he uses them for food, clothing and as beasts of burden;
> Resolved to limit the use of animals for experimental and other scientific purposes, with the aim of replacing such use wherever practical, in particular by seeking alternative measures and encouraging the use of these alternative measures;
> Desirous to adopt common provisions in order to protect animals used in those procedures which may possibly cause pain, suffering, distress or lasting harm and to ensure that where unavoidable they shall be kept to a minimum"

The Convention defines as animal:

> any live non-human vertebrate, including free living and/or reproducing larval forms, but excluding other foetal or embryonic forms" and states that animals can be used for the following purposes:
> a
>   i   avoidance or prevention of disease, ill-health or other abnormality, or their effects, in man, vertebrate or invertebrate animals or plants, including the production and the quality, efficacy and safety testing of drugs, substances or products;
>   ii diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, vertebrate or invertebrate animals or plants;
> b   detection, assessment, regulation or modification of physiological conditions in man, vertebrate and invertebrate animals or plants;
> c   protection of the environment;
> d   scientific research;
> e   education and training;
> f   forensic inquiries.

The European Directive 86/609/EEC

The European Directive, of which the complete title is "The Council Directive on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes" was adopted on the 24th of November 1986. It is composed of 27 articles and 2 annexes; annexe I lists the animal species most frequently used for research purposes annexe II is identical to the Appendix A of the European convention.

The Directive defines as animal "any live non-human vertebrate, including free-living larval and/or reproducing larval forms, but excluding foetal or embryonic forms" and allows the use of laboratory animals for the following purposes:

(a)
the development, manufacture, quality, effectiveness and safety testing of drugs, foodstuffs and other substances or products:
(i)
for the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality or their effects in man, animals or plants;
(ii)
for the assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
(b)
the protection of the natural environment in the interests of the health or welfare of man or animal.

European Member States have subsequently enforced the European Directive 86/609/EEC through national legislation which in some cases results to be stricter the Directive its-self. Differences concern the animal species covered by the Directive, the purposes for which animal research can be performed and the conflict of interest between the researchers and those in charge of guaranteeing the animal welfare. Moreover, according to the form of state of European Union Countries, the national Authority responsible to verify the provisions of the Directive changes as well.

National legislation in Europe

France

In France the Competent Authority is The Ministry of Agriculture. The French legislation, *Décret* 87-848 *relatif aux expérience pratiquée sur les animaux* of 19th of October 1987, allows the use of animal research for military purposes and in this specific case the competent Authority is the Ministry of Defence. This is stated by articles 20, 21 and 22 which read:

"Art. 20 (modifié par le décret n° 2001-464 du 29 mai 2001). - Par dérogation aux dispositions des articles 5 et 10 du présent décret, le ministre chargé de la défense est seul compétent pour recevoir et pour instruire les demandes d'autorisation d'expérimenter et pour accorder ou refuser les autorisations lorsque les expériences envisagées mettent en cause le secret de la défense nationale. Les autorisations sont données par le ministre chargé de la défense dans la limite des expériences nécessaires aux recherches qui relèvent de ses attributions. Elles peuvent être retirées discrétionnairement.

Art. 21 (modifié par le décret n° 2001-464 du 29 mai 2001). - Par dérogation aux dispositions de la section II du chapitre III ci-dessus, le ministre chargé de la défense est seul compétent pour agréer, dans les conditions qu'il détermine, les établissements d'expérimentation relevant de ses attributions.
Art. 22 (modifié par le décret n° 2001-464 du 29 mai 2001). - Par dérogation aux dispositions de la section III du chapitre III ci-dessus, la déclaration d'un établissement d'élevage ou de fourniture d'animaux destinés à des établissements d'expérimentation relevant du ministre de la défense nationale est faite à l'autorité militaire"

Germany

Since Germany is a federal republic the competent Authority for authorising the use of animal for scientific purposes is the Land and this may cause regulatory differences between various "Land". Contrary to France, the German legislation, The German Animal Welfare Act, prohibits the use of animal research for military purposes, as it is clearly stated in article 7 "(4) *Experiments on animals to develop or test weapons, ammunition and related equipment shall be prohibited"*. Germany has also solved the conflict of interest between the researcher and the person in charge of guaranteeing the animal welfare. In fact article 8b reads as follows:

(1) Heads of institutions, where experiments are conducted on vertebrates, shall appoint one or more animal welfare officers and notify each appointment to the competent authority. The notification shall also indicate the position and powers of the animal welfare officer under paragraph 6, third sentence of this Article.

(2) Only persons who have completed university studies of veterinary medicine, medicine or biology (spezializing in zoology) may be appointed as animal welfare officers. They must possess the expertise and reliability needed to discharge their tasks. In individual cases the competent authority may grant exemptions from the first sentence.

(3) The animal welfare officer shall be obliged:
   1. to ensure that the provisions, conditions and requirements shall be observed in the interest of animal welfare;
   2. to advise the institution and the staff involved in animal experiments and the keeping of laboratory animals;
   3. to give his opinion on each application for authorization to conduct an experiment on animals;
   4. to work towards the development and introduction of procedures and means for avoiding or reducing experiments on animals inside the institutions.

(4) If the animal welfare officer conducts an experiment himself, another animal welfare officer must supervise the planned experiment.

(5) The institution must support the animal welfare officer in the performance of his tasks and inform him of every planned experiment so that he can carry out his duties without restriction.

(6) The animal welfare officer shall not be bound by any instructions when fulfilling his tasks. He may not be discriminated against because of the performance of his tasks. His position and powers shall be laid down by statute, by internal instructions or in a similar form. In the process steps shall be taken to ensure that the animal welfare officer can voice his proposals or reservations directly before the decision-making body in the institution. If several animal welfare officers are appointed, the scope of duties of each shall be clearly defined.

Italy

In Italy the European Directive 86/609/ has been given effect with the *Decreto Legislativo* 116/1992 and the Authority controlling the implementation of the provisions of the above mentioned decree is the Ministry of Health. Differently from Directive 86/609/EEC, *Decreto Legislativo* 116/1992 states that the specific procedure for authorising an experimental protocol depends on the species used, the level of suffering to which the animals will be subjected, and the purpose of the research (Articles 7, 8, and 9). Based on these factors, the Principal Investigator must specify on the application form whether the experimental protocol will be evaluated as requiring "communication" or "authorisation". "Communication" is required when the experiments are to be performed on vertebrates other than those specified in Article 8 (i.e. species other than dogs, cats, non-human primates, and species protected by the Washington Convention), yet only when the procedures do not cause pain, suffering, or lasting harm, and

when they are not part of teaching activities. In these cases, only the Ministry of Health is involved in the evaluation process. The term "communication" refers to the fact that the researcher is merely "communicating" to the Ministry that the research is going to be performed, although this term can be considered as somewhat of a misnomer, in that the protocol must in any case undergo evaluation. When the experiments are to be performed on the species specified in Article 8 or on any vertebrate for teaching purposes, or when the procedures can cause suffering, stress, or lasting harm, the evaluation process is referred to as "authorisation". In these cases, the Ministry of Health is required to contact the Istituto Superiore di Sanità (ISS; the National Institute of Health in Italy), which plays a key decision-making role in the evaluation process.

United Kingdom

In the United Kingdom the Competent Authority for is the Home Office. The British legislation, Animals (Scientific Procedures) Act, as far the animal species are concerned is stricter than the Directive and covers some invertebrates as well. In fact it was amended in 1993 as follows in "For the purposes of the Act "a protected animal" shall include any invertebrate of the species Octopus vulgaris from the stage of its development when it becomes capable of independent feeding." This is due to the fact that octopuses have been demonstrated to possess a complex nervous system.

Conclusions and European perspectives

Considering the need to improve laboratory animal welfare and to further harmonise the regulations amongst European Union Member States, it appear clear the necessity to revise the European Directive. Moreover it has to be taken into account that since the European Directive was adopted in 1986, there was an increase in new scientific techniques, such as animal cloning and the use of transgenic mice, which are not regulated by the European Directive.

For this reason, under the guidance of the European Commission, an expert working group has been appointed to analyse current problems and find possible solutions.

Some of the topics to be focused on will be the possible inclusion into the European Directive of invertebrates, such as cephalopods and 'arthropods, and foetal and embryonic forms of vertebrate animals, the authorization procedures and the appointment and independence of the animal welfare officer.

It is worth noticing that some of these matters have already been solved by some national regulations.

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ETHOLOGICAL INDICATORS OF THE WELL-BEING OF NON-HUMAN PRIMATES

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Introduction

In this paper we will present a series of behavioural indicators that can help to characterise the state of welfare of experimental animals. We will mainly refer to non-human primates, and in particular to the common marmosets (*Callithrix jacchus*), which is our study species. However, we believe that the subjects offered here can be valid in general terms as well, and then to be applied to other experimental species. We are well aware of the fact that, in order to have a comprehensive picture of the welfare state of individual, behavioural parameters only are not enough. Therefore, at the end of this paper, we will briefly mention physiological parameters that need to be taken into consideration. However, the focus of the contribution will be on behaviour.

What is animal welfare?

When discussing animal welfare it is essential to offer a definition of the concept, in order to better focus the arguments proposed. Unfortunately, in the case of animal welfare, this is a rather difficult task. As a matter of fact, animal welfare is a very broad term, and a definition universally accepted to describe it does not exist. However, an element, which is important to underline, is that animal welfare is a relative concept. It is not correct to affirm that a certain individual "does or does not have state of welfare"; rather, individual experiences different degrees of welfare, and researchers should actively work to improve such a state (1).
Besides agreeing on a common definition of animal welfare, researchers discuss also on the relative importance of different factors, which better indicates the level of welfare. Duncan and Fraser presented three different schools of thoughts: i) welfare depends on animal feelings; ii) welfare depends on the functioning of the biological machine; iii) welfare depends on the respect of the "nature" of the animals (2).

In the first case the level of welfare will be compromised by subjective negative feelings such as, for example, fear and frustration; conversely, experiencing comfort and pleasure will cause an improvement of the animal's state. The problem with this approach is that feelings, differently from other individual characteristics, cannot be observed directly, and are open to the observer's subjective interpretation. However, we can postulate that these unobservable phenomena are caused by certain observable circumstances, and do affect the manifestation of certain behaviours. For example, we are able to categorise certain type of vocalisations belonging to the repertoire a certain animal species, to an aversive or stressful condition (3).

Other authors propose proper physiological functioning as a valuable measure of the degree of animal welfare. Therefore, disease, injuries and malnutrition will cause a decrease in the level of welfare, whereas growth and a good rate of reproduction will be the cause of a satisfactory

state of welfare. On one hand, one of the advantages of this point of view is that it is easier to scientifically substantiate it than, for example, the feelings approach; on the other, the link between biological functioning and welfare is not always so clear. For example, an increment of the reproduction rate or milk production of dairy cows is not necessarily an indication of improved welfare.

Finally, some researchers argue that the more the animal in captivity is behaviourally different from the wild counterpart, the more its state of welfare is compromised. Therefore, animals in captivity should be free to perform their natural behavioural repertoire (4). Obviously, in terms of the welfare of captive animals, this is a very appealing point of view, but it runs into conceptual difficulties. For example, the concept of "natural behaviour" should be better explained. Furthermore, is this implying that we should provide captive animals with aversive stimuli, such as the presence of predators or the conditions for social disputes, because these are integral part of their wildlife? Is this in contradiction with the very concept of welfare? (5, 6).

In relation to the three approaches to animal welfare outlined above, it is rightly argued (2) that the best approach is a combination of the three. As a matter of fact, there is also a considerable overlap among the different points of view. For example, giving the possibility to an animal to perform natural behaviours can promote its biological functioning (7).

To conclude, we can say that to promote in an animal a good level of welfare can also mean to assure that its needs are satisfied. Curtis, for example, has proposed that animal welfare depends on a "hierarchy of needs": i) "physiological needs"; ii) "safety needs"; iii) "behavioural needs" (8). Later, other authors have identified "life-sustaining needs" as the most important ones, followed by "health-sustaining needs" and then "comfort sustaining needs" (9). However, objections to this hierarchical approach to animal needs have been presented (10).

Ethological indicators of a satisfying level of welfare

Time budget

A behavioural observation is an indirect measure of how an animal "feels" in a certain moment, but nevertheless it represents a very useful methodological tool in animal welfare science. As we have seen above, the naturalistic approach to welfare proposes a comparison between the behavioural repertoire of an animal in captivity with the repertoire of the same species in the wild. If the similarities between the two repertoires are relevant, it is assumed that the degree of welfare is satisfactory. Despite the conceptual difficulties outlined above, the comparison between captive and natural behavioural time-budget can be really useful. For example, non-human primates spend considerable time in the wild looking for, processing and consuming food (11, 12). This time in captivity can be drastically reduced: usually, the food is provided everyday more or less at the same time, it is already prepared and easy to consume. This kind of situation prevents an important series of behavioural patterns to be performed, and detracts from the animal the possibility to control and actively approach its own environment. The need to have a certain degree of control on a captive environment is considered to be fundamental to maintain a good level of welfare (13). In the colony of common marmoset housed at the Istituto Superiore di Sanità, in the Department of Cell Biology and Neuroscience, we have introduced a device called the "puzzle-feeder", which require a certain amount of work and time in order to extract food from holes of different diameters. The results of our experiments with the puzzle-feeder showed that the monkeys increased the time spent in food-related activities, during the diurnal time-budget, from the 19% to the 25%. This outcome can be reasonably considered as a move toward a higher degree of welfare (14).

Play

Although the explanation on why animals play is illustrated by several different theories (see for example 15), the presence of playful behaviour in colony captive animal is considered a sign of a general good level of welfare. Play, behaviourally speaking, can be considered a luxury: animals play when they do not have to worry about feeding, predators, threatening social dynamics, physical health. In other words, the motivation to play arises when essential needs to survive are satisfied. Hanuman langurs (*Presbytis entellus*), an Old World primate species, have been observed to play only when satiated (16).

The performance of play is also thought to be reinforcing in itself, and possibility to play as a reward has been successfully utilised in conditioning experiments in different species of animals, including rodents (17). Play behaviour occurs mainly in juvenile animals. In 720 hours of observations of eight adult common marmosets only few incidences of social play between adults were observed (18). Therefore, the frequency of play behaviour occurring in a captive group of primates depends, but not only, on the age composition of that particular group. Social play is often accompanied by particular facial expressions, particular postures and typical patterns. For example, in "rough-and-tumble" play, the role between attacker and attacked is exchangeable, and different types of playful activities can be showed during the same play bout (19).

In common marmosets, like in other primate species, open-mouth expression is considered an invitation to play, as well as lying on a hard surface with the back of the body (18). In the same species the introduction of environmental enrichments, such as hanging pipes or water crates, has been observed to elicit play (20).

Social grooming

Social grooming, or allogrooming, is considered to be important in the development and maintenance of social bonds and group cohesion in primates (21). It consists in the ritualised cleaning of the fur of an individual by a conspecific. The social function of this behaviour is particularly relevant in species whose social system is characterised by more or less stable social hierarchies, such as macaques and baboon species (20, 21). An increase in the frequency and duration of social grooming bouts is likely to be indicative of a good social environment existing in a captive group of non-human primates. However, allogrooming is one of those behaviours which have to be analysed within the context in which are observed, in order to understand its meaning in term of welfare. As a matter of fact, allogrooming has been shown to reduce tension between individuals (22). It slows down the hear-rate of the receiver, and induces the release of endogenous opioids. Therefore, an unusual increase of grooming activities in a group of primates could indicate high level of tension in the colony, due to the recent occurrence of a stressful event. For example, in a group of tufted capuchins, cage-mates were observed to groom the dominant male when he displayed stressful behaviour, as a consequence of an aversive experience. These kinds of consideration obviously require an adequate knowledge of the general behavioural patterns of the colony under observation, with a clear idea about the baseline level of allogrooming occurring in the colony under observation. Having said that, Stevenson and Poole have noticed that, in common marmoset, the posture utilised to elicit grooming from a cage-mate is different depending on the context. A stretched out posture on the back or on the side is more likely in amicable interactions, when the animals are at ease and not stressed (18).

Vocalisations

Different researchers have proposed that the study of vocalisations may be a useful method for investigating physical and psychological functioning, and their relation to welfare. This can be true for different species housed in research laboratories. In particular, non-human primates are characterised by complex vocal repertoires.

Vocalisations can be monitored and recorded in a non-invasive way, and provide an indirect indication of the internal state of an animal, in relation to a certain captive condition or event. In the case of the common marmoset, already in 1968 Gisela Epple has described many different types of calls of this primate species (23). Among the calls suggesting a state of comfort, we can cite here the "food calls". These are calls emitted in the presence of a preferred food item (24), and are interpreted as the expression of a satisfactory feeling, as well as a way to attract family-members to the source of food (26). Epple described this particular type of vocalisation as: "faint, high pitched notes, which vary in duration and intensity… The vocalisation may last for several minutes and sounds much like the faint chirping of birds". Such calls, or very similar ones, are also emitted in the evening when members of a group are huddled together, and similar calls can be heard during playful and affiliative behaviours, such as hugging. Other calls, described as "contact calls" are heard during normal activities in a group of captive marmoset, when all of the members are in view of each other. Also these calls are generally interpreted as a sign of social comfort (25). The "contact calls" seem to have an inverse relationship with the emission of "phee calls", often heard when cage-mates are not in view of each other. The transition from one call to the other can give indication of the emotional state of the caller, in relation to its social needs.

Ethological indicators of an unsatisfying level of welfare

Many studies have been carried out to determine the kinds of behaviours associated with an unsatisfying level of welfare. These behaviours vary depending on the type and severity of the stressful situation experienced by that particular individual. In general, the first response to a stressful situation is called the "fight-flight response", characterised by an active behavioural response. If the stressful situation persists, the animal could fall into a depression-like mode, mediated by a series of hormones of the pituitary adrenal cortical system and characterised by a secretion of glucocorticoids (26).

Perhaps the most revealing sign of a decreased level of welfare of captive animal is a change in the frequency and ways of the basic behavioural profile of a particular individual. An unwell animal usually vocalizes more than the usual, refuses human contact, moves and rests in unusual postures. These are banal factors but, nevertheless, they require knowledge of the characteristics of the basal ethogram of that particular animal colony utilised for laboratory studies.

We will now briefly mention behaviours that can give us some indication of a decreased level of welfare.

Locomotion

A change in the level of locomotory activity has been observed to be correlated with the presence of stressful events. For example, in the common marmoset it has been shown that the rate of locomotion increased for four hours following a stressful event (27). In a related species (black tufted-ear marmoset, *Callithrix penicillata*) researchers, observing the effects of

anxiolytic drugs on the reaction to the presence of predators, have concluded that a high level of locomotion could be an index of stress in marmoset monkeys (28).

In other species of non- human primates as well an increase in locomotion in the presence of predators or after separation from group-mates has been observed (29, 30). However, in other cases, such increase does not necessarily indicate a decrease in the level of welfare. As a matter of fact, if we provide a group of monkeys with a larger cage, equipped with physical enrichments, and with food scattered on the ground rather than concentrated in few fixed places, most probably an increase in locomotion will be recorded. It is hard to affirm that in this case such increase is due to a decrease in the general level of welfare. Another similar example could be represented by the effort in shaking an individual off from a state of tonic immobility, providing some sort of enrichment (31): also in this case the increase of locomotory activity can not be related to a decrease in the degree of welfare experienced by that particular individual. So, an increase in locomotion its beneficial for those individuals who are initially inactive and apathetic. But again, in some cases, the introduction of novel objects could create a state of anxiety, resulting in more pacing across the cage. Therefore it is necessary, in order to correctly interpret the behavioural variation observed and relate it to the level of welfare, to know the baseline behavioural values of the animals observed, and the context in which the variation has occurred.

Abnormal behaviours and stereotypes

Certain behaviours are easier to associate with an unsatisfying state of welfare. For example, we can cite here self-biting; the excessive self-licking or licking portions of the physical environment; polidpsya, tonic immobility or iper-activity, persistent biting or sucking body parts of cage-mates, such as the tail (32). Furthermore, stressful situations can be the cause of substitutive behaviours, in which an individual is unsure between two different behaviours to adopt, and therefore displays displacement activities such as, for example, self-scratching (33).

Behavioural stereotypes are important manifestations of the poor state of welfare experienced by an animal. There are many different types of stereotyped behaviours, depending also on the type of stressor, and have been the subject of several studies in different areas of research. These behaviours have been described as: "a behaviour pattern that is repetitive, invariant and has no obvious goal or function" (34). Generally, these behaviours appear when an animal is motivationally frustrated: it wants to do something, but features of the environment do not allow the manifestation of that particular behaviour. A clear example is the pacing back and forth in zoo cages by mammals used to roam in nature in large spaces, such as bears or different species of felids. When stereotypes appear, it means that something is wrong with the environment, and we are too far from a natural situation. In other words, the animal is disturbed because the situation in which it finds itself in captivity is too different from its own evolutionary eco-ethological niche. An important characteristic of a stereotyped behaviour is that its manifestation can continue even if the cause of the frustration is removed (34). Therefore, a stereotypy can be the indication of a past stressful situation, rather than something happening in that particular moment. Some authors suggest that behavioural stereotypes become a problem needing intervention, when they occupy more than the 40% of the time budget of an individual. Stereotypes can cause injuries, which can predispose to infections (35). For what concerns common marmosets, the most frequent type of stereotyped behaviours are cage circling and pacing. These stereotypes are likely to be shown by individuals who have been suffering from social isolation especially during the early stages of development. These disturbances are unlikely to disappear when the individuals reach adult age. This also means

that the quality of the environment in which a young individual grows up it is essential for its welfare state as an adult (36).

Social interactions

Sociality is an essential characteristic of the behavioural repertoire of non non-human primates (37). The kind of social interactions observed among cage-mates can give an indication of the general state of welfare of a colony of captive non-human primates. For example, members of a social group of marmosets separated for one hour will show a significant increase of the frequency of affiliative behaviours, in order to reduce the stress caused by the experienced stressful event (38). This result does indicate that separation is stressful, and sociality must be an essential part captive primates housing management.

Hence, it is crucial to follow closely the state and the development of social relationships within a group of captive monkeys. For example, variations in the hierarchical organization of the group could occur, with potential danger for the physical incolumity of subordinate individuals in a confined environment. In our laboratory, housing a colony of common marmosets, we have observed in the past 13 years about a dozen of expulsions of single individuals from their social groups. These events are relatively unpredictable, but they appear to be related, among other factors, with the number of individuals housed in a confined space of certain dimensions. Furthermore, forming new social groups is difficult, and demands a high level of caution. In black tufted-ear marmoset, the presence of new same-sex conspecifics resulted in an increase of aggressive and agonistic behaviours, accompanied by an increase in urinary cortisol (39). New individuals introduced in an established social group can result in high level of stress for the introduced individual. Therefore, on one hand, socially isolated animals can develop immunitary problems, and primates kept alone show significantly more often behavioural abnormalities than primates group housed; on the other, too many individuals in a confined space can be cause of acute social stress for certain individuals. Beyond physical aggressions, a subordinate member of a social group can develop severe depression-like symptoms just because in constant view of the dominant cage-mate. Higher concentration of cortisol has been observed in subordinates different species of primates, such as talapoin monkeys (*Miopithecus talapoin*) (40), and olive baboons (*Papio anubis*) (41). Sociality guarantees a good level of welfare, but it has to be monitored and followed constantly and with attention.

Physiological parameters

We have previously mentioned that behavioural parameters only can give just a partial picture of the state of welfare of a particular individual. It is therefore important to consider physiological parameters as well. Here we will just mention few of these parameters.

The weight of individuals should be constantly monitored. Monkeys in captivity gain weight. However, pain and stress can lead to a decrease in weight. For example, it has been observed that infant common marmosets separated from their parents every day for about two hours during the first month of life limited their growth rate, compared to controls (42). Therefore, if variations of weight do occur routinely, potential stressful situations in the animal colony have to be checked for.

Heart rate can give us important indications on the welfare state of an individual. Heart rate frequency is linked with the amount and the type of activity performed during the day.

However, a telemetry study carried out on common marmosets showed that heart rate frequencies were also related to the level of disturbance experienced by the animals during the day. Therefore the interpretation of heart rate frequency variation, and its relation with animal welfare, must be considered in relation to the specific context of the moment.

Obviously, hormonal levels are a very important indication for the general state of welfare in a colony of captive animals. For example, the amount of circulating cortisol is commonly used as a measure of the condition of stress experienced by a particular individual. For what concerns primates, it must be notice that New World monkeys have a basal level of cortisol 25 times higher than Old World monkeys and humans (43). The concentration of circulating cortisol has been observed to increase in a stressful situation, and a positive correlation between the presence of this hormone and severity and duration of the event has been recorded. Cortisol level can be measured in the blood, urine, feaces, saliva, and can be measured five minutes after the potentially stressful event (44). The most common methodology is to measure the concentration of this hormone in the blood, but recently the less invasive methodology utilising saliva is becoming increasingly popular (45).

Conclusions

As researchers working with animal models, we have an ethical and scientific obligation to guarantee to the experimental animals the best possible conditions of welfare. In order to do so, we have to find ways to identify the state of welfare of a particular individual in that particular moment of its captive life, in relation to past and present environmental events. It is absolutely necessary to know the animals we work with. This knowledge, especially in the case of non-human primates, requires both an attention towards the needs of a particular individual as well as an understanding of the to-ecological characteristics of the species under study. In this respect, a comparison between the behaviour of the captive animals and the behaviour of wild conspecifics can be very useful. However, we also have to keep in mind that generations of life in captivity could have changed some of the behavioural needs of our animals. For example, in our colony of captive marmosets we have not observed a motivation to work in order to obtain food, that we would have expected on the basis of the time this species spend in the wild searching for and processing food. Furthermore, different captive colonies of the same primate species could differ in terms of potential events influencing their degree of welfare, depending on the experimental and management history of that particular colony. "Know your animals!" must be the requirement for any researcher intended to reach a convincing level of quality for both the scientific values of the data obtained, and the level of welfare of the experimental animals.

Acknowledgements

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RELATIONSHIP BEETWEEN TRANSPORT AND ANIMAL WELFARE IN AVIAN AND RABBIT SPECIES

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Introduction

It is well known that events which occur before animal slaughtering (feed and water withdrawal, crating, transportation, abattoir lairage) are critical for several aspects for animal welfare, but also for liveweight loss, slaughtering yield as well as meat quality and safety (1-4). Several regulations dealing with the protection of animals during transport have been adopted since 1977 by European Union (Table 1).

Table 1. **Animal welfare during transport: regulations and scientific reports adopted by the European Union and recommendations adopted by the Council of Europe**

Title	
European Directive (D) and Regulations (R)	
International transport of animals	D 77/489/ECC
International transport of animals	D 81/389/ECC
Protection of animals during transport	D 90/425/ECC
Protection of animals during transport	D 91/426/ECC
Protection of animals during transport	D 91/628/EC
Protection of animals during transport	D 93/119/EC
Protection of animals during transport	D 95/29/EC
Criteria for staging points	R 97/1255/EC
Protection of animals during transport	R 98/411/EC
Protection of animals during transport	D 99/575/EC
Protection of animals during transport	R 2005/1/EC
Council of Europe recommendations	
European convention for the protection of animals during international transport	ETS 65
Scientific reports of European Union	
Transport of farm animals	VI/3404/92 - EN
Standards for the microclimate inside animal transport road vehicles	SANCO/B3/AW/R13/1999
Standards for the microclimate inside animal transport road vehicles	EFSA-Q-2003-085
The welfare of animals during transport	EFSA-Q-2003-094

In these documents several points are addressed, and specific attention is paid to long journeys. The main concerns of the regulations with impact on poultry and rabbit species deal with: 1) animal density in crates and crate height for poultry species (Table 2 and 3) (no recommendations on space requirements are given for rabbits); 2) design of the truck: in particular, the animals should be able to drink and eat during the whole journey in case of transport lasting more than 12 h (for chicks of all species, this period can be prolonged until 24 hours in case of transport completed within 72 hours after hatching); precautions shall be taken to limit urine and faeces falling on the animals placed underneath; the temperature should range between 5 and 30°C inside

vehicles naturally ventilated ±5°C depending on the outside temperature; sufficient space shall be provided to ensure that there is adequate ventilation above the animals when they are in a naturally standing position, without on any account hindering their natural movement; 3) training of workers; and 4) all transport companies should have a license that can be withdrawn if they do not comply with the requirements. A further general provision for mammals is particularly relevant for the transport of young rabbits who "shall not be considered fit for transport if they are new-born mammals in which the navel has not completely healed". In fact it is a common practise to move young breeders 2-4 days *post*-parturition from genetic centres to commercial breeder farms, but actually no scientific data are available on the time in which the navel has completely healed. All these constraints are quite heavy and put pressure on the industry. Their application under practical conditions is still under discussion. New documents which take into account new scientific knowledge will be necessary in the future.

Table 2. Densities applicable to the transport of poultry in containers (R 2005/1/EC)

Category	Area (cm^2)
One day-old chicks	21-25 per chick
Poultry other than day-old chicks (kg):	
< 1.6	180-200 per kg
1.6 to < 3	160 per kg
3 to < 5	115 per kg
> 5	105 per kg

Table 3. Height inside the transport containers applicable to the transport of poultry (Council of Europe recommendations 90.6)

Category	Height (cm)
Day-old chicks	10
Poultry other than day-old chicks (kg):	
≈ 1	20-23
1 to 4	24-33
> 4	>34

More specific provisions for poultry and rabbits are foreseen considering the recent opinions of the European Food Safety Authority (EFSA). The EFSA Scientific Subcommittee on Animal Welfare has recently adopted a scientific report for the European Commission also including the dissertation of animal welfare in rabbits (EFSA-Q-2003-094). This paper is aimed at making a short general statement of the recent studies carried out on animal welfare during transport in avian and rabbit species.

Welfare during transport of avian species in commercial systems

Chicken broilers, turkeys and laying hens will be transported at least twice during their lifetime, first from the hatcheries to farms as "day old" chicks, and second from farms to abattoirs (Figure 1). Laying hens will also be transported to the egg production unit at the beginning of egg laying period. Major sources of welfare concerns in the transport of broilers,

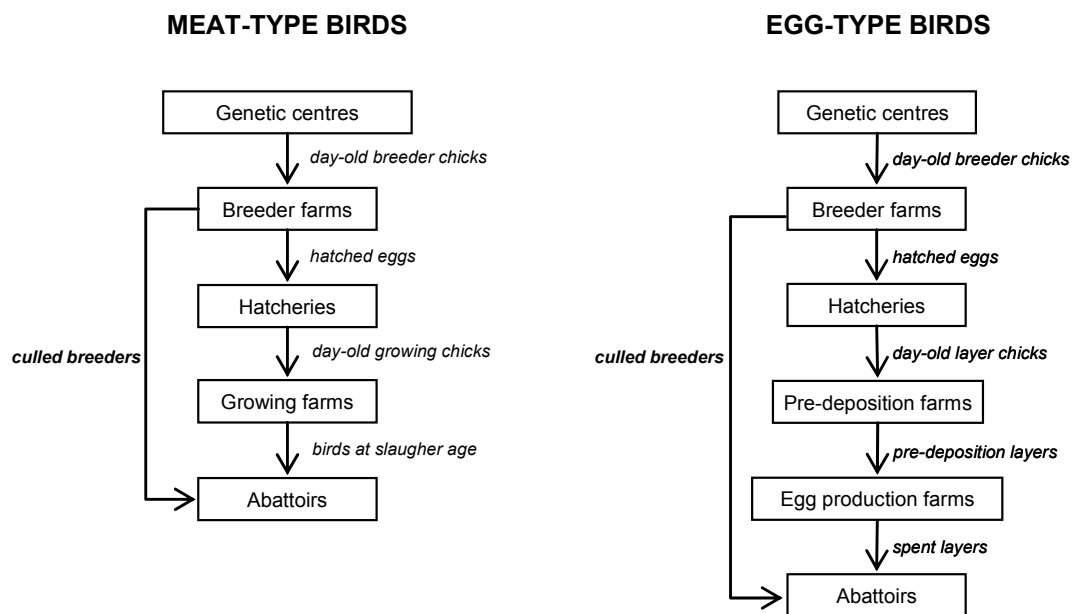turkeys and hens are the process of catching and crating birds and the thermal micro-environment in transit.

MEAT-TYPE BIRDS   **EGG-TYPE BIRDS**

Figure 1. Flow-chart of production chains of meat and egg-type birds

Preparation of animals to transport (catching and crating)

A more careful bird handling to reduce trauma has been reported as crucial factor to reduce mortality and trauma as mainly broken and dislocated bones, bruising and haemorrhages (10, 11, 12).

Catching of broilers is usually by the legs and operatives may carry up to five birds, each held by one leg, in each hand. The potential for trauma is therefore considerable. In particular, it may lead to dislocations of the femur at the hip joint (13). Also in turkeys, it has been evidenced that during loading, birds may suffer death, bruising, broken bones, torn skin and other physiological stress (14). Spent hens are transported to processing plants when they are at the end of laying period and preslaughter mortality and physical damages have been observed to be very critical. This is mostly due to injuries formed during cages removal and crating because osteoporosis in laying hens leads to loss of structural bone and increased incidence of fracture at various skeletal sites by the end of the laying period (15). Gregory *et al.* (16) found that 10.2% had old and healed breakages (principally the furculum, ulna and humerus) and a further 16.5% breakages occurred during depopulation and transport (most frequently the pubis and the keel). At this stage, Gregory and Wilkins (17) observed that 31.4% of birds had broken bones, rising to 45.3% following removal from transport crates and hanging on to shackles prior to stunning.

There is variation between the proficiency of different catching teams implying that training of operators would decrease trauma. Lifting and carrying domestic fowls by both legs, in comparison with one leg, reduce stress and trauma. Mechanical catching systems for broilers have the potential to reduce trauma, stress and mortality. The machines most commonly used in

Europe are of the sweeter-type with a vertical three-rotor pick-up head with soft rubber fingers. However, contradictory results have been found when comparing the results on dead-on-arrival (DOA) and injuries prevalence in birds harvested by manual *versus* machine catching (18).

Crate size, space allowance, floor type

It appears to be little or no research on appropriate stocking densities for broilers or hens during transport. EFSA-Q-2003-094 report suggests the use of the generalized formula for defining minimum floor space requirements during transport (Area (m^2) = 0.021×weight $(kg)^{0.67}$) based on the recommendations from New Zealand and Canada for broilers. For a 2 kg broiler the floor space is equivalent to 60 kg per m^2 and for one weighing 2.5 kg it is equivalent to 64 kg per m^2. Reducing stocking densities by about 10% in hot weather will mitigate the deleterious effects of high temperature on mortality.

Regarding with the crate floor, a further critical point is the need to prevent the transfer of faeces from upper crates in a stack to those below without compromising natural ventilation during transport. Some new crate models are present on the market which allow better air movement on the truck, without causing problems with droppings from upper cages.

Fasting

Broiler fasting before catching and transport is a standard management practice. Feed withdrawal before slaughter allows emptying of the digestive system and reduces the incidence of faecal contamination during processing. The optimal feed withdrawal time must be long enough to allow sufficient gastrointestinal tract clearance in the event of accidental rupture during evisceration, but also short enough to reduce bile contamination and weight loss and to be acceptable from the welfare point of view (19, 20). A feed withdrawal period of approximately 8 to 12 h is recommended. Under commercial conditions, no systems for feeding and watering during transport or at lairage are currently available. Deprivation of feed and water have been shown to cause depletion of glycogen in the liver, which is the primary store available for maintaining blood glucose levels, as early as after 3 h of feed withdrawal (2). In living condition liver glycogen is reduced to negligible levels within 12 h of deprivation, when birds may feel very hungry. During transport, metabolic rates are much higher so food reserves are depleted much faster.

Fasting is known to influence a whole array of metabolic processes including a shift from anabolism to catabolism and from lipogenesis to lipolysis. These adaptive changes are mediated by many metabolic hormones. In chicken (21, 22) it has been observed that fasting reduces circulating triiodothyronine (T3), insulin, and insulin-like growth factor-I (IGF-I) levels, whereas plasma glucocorticoid levels are elevated (22, 23).

Transport

Transport of broilers leads to an average mortality of about 0.3-0.4% and mortality rate increases with the length of the journey. In an Italian recent extensive survey, Petracci *et al.* (24) observed a preslaughter mortality of 0.35 and 0.38% in broilers and turkeys, respectively, while a higher mortality rate was found in spent hens (1.22%). These data are quite higher in respect to those reported by Warriss *et al.* (25) who indicated dead-on-arrival (DOA) bird prevalence of 0.126% in UK. Moreover, Nijdam *et al.* (12) considering 1,907 Dutch and German broiler flocks slaughtered in 2000-2001 at a single processing plant found a 0.46 average DOA

percentage. Weeks and Nicol (26) also suggested a conservative figure for overall average mortality of broilers as 0.3%. However DOA prevalence in poultry is higher if compared with preslaughter mortality found in cattle (0.05%) and swine (0.01%) (27) as well as rabbits (0.08%) (28).

Petracci *et al*. (24) also found that the season significantly affected the DOA incidence in all considered poultry categories. Preslaughter mortality in summer was found to be dramatically higher in broilers (+43%), turkeys (+59%) as well as spent hens (+42%) than mortality observed in autumn, winter and spring. A similar seasonal trend was observed by Warriss *et al*. (25). These studies confirm that heat stress is a major factor contributing to the death of birds during preslaughter time, while low ambient temperatures appear generally to be less important as previously stated by several authors (2, 29, 30). Barontini *et al*. (31) evidenced that DOA are caused more by *ante mortem* stress than preexistent pathologies. They found that the main causes of preslaughter mortality are acute (14%) and congestive (37%) heart failure followed by trauma (32%). This agrees with Bremner and Johnston (32) who indicated that heart failure was accounted to be 47% of the deaths. Both environmental temperature and humidity are important factors affecting metabolic exhaustion and dehydration which can cause heart failure (33). An important method of losing heat in poultry at high temperatures is by evaporative cooling from the respiratory tract. The birds pant to facilitate this. At high humidity evaporative mechanisms become less effective, or ineffective (30). A high temperature and humidity during preslaughter time (in the broiler house during catching and loading, on the vehicle during transport and in the lairage area at the abattoir) may be a part of the explanation for greater mortality during summer (12). Mitchell and Kettlewell (30) linked physiological stress to thermal microenvironment during transport with a combined index called "apparent equivalent temperature" (AET). This parameter combines the dry-bulb temperature and vapour density, which can be calibrated by physiological indicators to give a measure of stress. An AET value <50°C is considered safe for the transport of poultry. Apparent equivalent temperature values between 50 and 70°C are potentially stressful if maintained for prolonged periods and may lead to some mortality. Values >70°C are considered stressful with a high risk of mortality. A crucial factor is that a close environmental control in the crates or modules on the vehicle is difficult, mainly because on most vehicles ventilation is passive and is impeded by the close stacking of adjacent crates. Moreover mortality is higher in consignments of broilers transported for longer time (10). For journey lasting less than 4 h the prevalence of dead birds was 0.156%, while for longer journey (up to 9 h) it was 0.283% an increase of about 80%. In a similar study, Petracci *et al*. (34) found that a shorter journey time (<3.5h) exhibited a lower mortality rate (0.24%) of birds in respect with longer time (>5h; 0.45%). Increased corticosterone levels after 1 to 3 h of transport, although ranging widely, indicate transport stress (35, 36). The recent adoption of new rules on long transport (R 2005/1/EC) should improve animal welfare conditions during transport. In fact, starting from 2009, all vehicles operating long transport must be equipped by devices able to maintain suitable environmental conditions (T and RH) avoiding extreme conditions.

Chicks transport

Breeding and growing day-old chicks are transported in plastic or paper boxes by air and road. EU regulations enforce that day-old chicks transport has to be completed within 72 hours after hatching. A recent study has shown that the thermoneutral zone for individual day old chicks is 30-32°C (5), so that in EFSA-Q-2003-094 report is recommended to maintain 25±1°C as environmental temperature at a relative humidity of 60-70% when commercial stocking densities (21-25 cm^2 per chick) are adopted.

Domestic fowl and turkey chicks can survive for several days following hatch due to the internal storage of excess yolk. Modern broiler genotypes have higher metabolic rates than older

breeds and use up the nutrient reserves stored in the yolk sac more quickly. Chicks of modern genetic strains are therefore not able to survive as long as the commercially reared breeds in past years without access to food and water. Some studies showed that nutrients were 90% depleted at 41 h and little uptake from yolk occurred after 48 h (6, 7, 8). In North America, a commercial product (high water jelly) has been marketed for long air transport of day-old breeder chicks. Xin and Lee (9) found that the use of this product allow to obtain chicks with more vitality and higher productive performances during the next growing period.

Welfare during transport of rabbit in commercial systems

Both breeder and growing rabbits are transported at least once during their life (Figure 2). In the recent scientific report (EFSA-Q-2003-094) adopted for the European Commission, the main concerns for the protection of rabbit welfare during transport are directed on: i) shape of the crates which should allow the rabbits to stand in natural position; ii) space allowance of a minimum of 0.06 m^2/rabbit; iii) adequate ventilation should be provided during transport to maintain the inside crate temperature within the range 10-20°C; iv) journey duration should not exceed the range of 8-12 h.

* The breeder and growing animals can be held in the same farm (“closed cycle”)

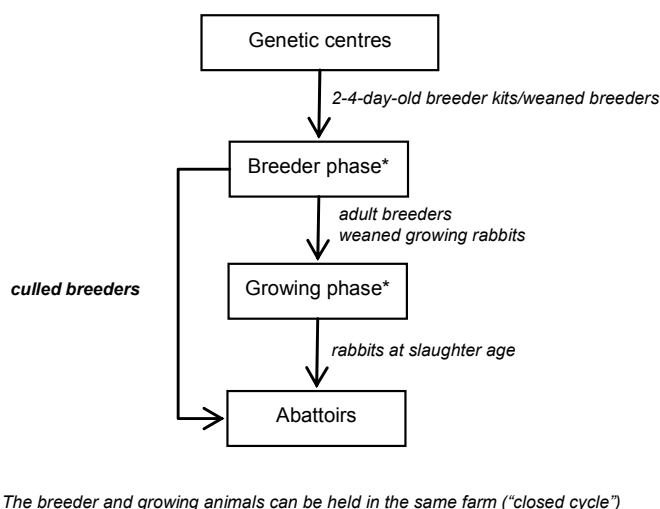**Figure 2. Flow-chart of production chains of meat-type rabbits**

Preparation of animals to transport (catching and crating)

Before transport to the abattoir, rabbits are removed from the growing cages and loaded into crates by hand. Loading can be carried out either inside the farm by placing the crates close to the cages or outside by moving the rabbits near to the truck and put them into crates standing on

the truck. In a comparative study under commercial conditions, it was showed that rabbits loaded into crates placed on the truck had a slight reduction (0.44%) in prevalence of carcass with loin bruises compared with those crated on the farm (37). Without regard of crating method, a more careful rabbit handling to reduce trauma has been reported as crucial factor to reduce preslaughter mortality and carcass defects as mainly haemorrhages, bruises and broken bones. The areas of the rabbits most frequently bruised are legs, thoracic muscles and the internal part of the loin region. These bruises are mostly not detectable in the live rabbit and become visible only during slaughtering after skin removal with detrimental effect on carcass quality (4).

Crate size, space allowance, floor type

Under commercial conditions, growing rabbits marketed for slaughter are generally transported in plastic wire crates. The dimension of the crates can be quite variable, however the standard crates measure $100-110 \times 50-60 \times 22-30$ cm (length×width×height). The number of rabbits loaded into crates varies according to animal weight and environmental conditions: 14-16 animals/crate for rabbits weighting 2.0-2.7 kg and 12-14 animals/crate for rabbits weighting 2.8-3.2 kg. The resultant commercial stocking density varied from 0.03 to 0.05 m^2/rabbit. As reported before, the scientific report EFSA-Q-2003-094 indicated a recommended stocking density of 0.06 m^2/rabbit. De La Fuente *et al*. (38) comparing rabbits transported at low (0.05 m^2/animal) and high (0.03 m^2/animal) stocking densities did not found any difference in physiological parameters related to stress. They suggested that other factors such as the height of the cage or the number of piled crates have to be taken into account when studying the effect of stocking density.

Regarding with the crate floor, Jolley (1) recommended adopting solid floors in transport crates to prevent the transfer of urine and faeces from upper crates in a stack to those below. There are several particular concerns about this aspect because ventilation is generally passive on vehicles and can be partially impeded by crates with solid floor. A further important practical concern is the higher difficulty to clean the crates with solid floor at abattoir after slaughtering in respect with those made of wire.

Fasting

During the period lasting between catching at farm and hanging at the abattoir line, rabbits are kept without feed and water. Moreover, feed can be removed before rabbits are caught to allow time for the evacuation of the intestinal contents. This practise may contribute to reduce the incidence of faecal contamination of the carcass which may occur during gastrointestinal tract removal as well as reducing stress during transportation, while there is less sensibility about this practise than for poultry. In this case, fasting should be referred to the total length of time rabbits are without feed before processing including the time in which the rabbits are in the farm without feed, as well as the time rabbits are in transport and in the holding area at the processing plant. Length of food deprivation is important because it affects animal welfare, but also commercial carcass yield (live weight losses), carcass contamination and product safety (pathogenic and spoilage bacteria) and quality (ultimate muscle pH) (1, 39). However due to caecotrophy, rabbits are usually considered to be very resistant to hunger (40). Of course the way in which transport affects the pattern of changes to gut fill depends on whether the rabbits are allowed free access to feed and water before crating. Crating and transportation can also cause the rupture of caecotrophy practice which leads to higher spillage and rabbit

contamination (1). Rabbits lose 3-6% of body weight during the first 12 h of fasting, increasing to about 8-12% at 36-48 h (4). Generally, weight loss is slightly lower if fasted rabbits are allowed access to water before crating (41). In the first 4-6 hours, weight loss in rabbits is mainly due to emptying of the gut, so carcass yield is not negatively influenced (42). After 6 hours, there is also a loss in moisture and nutrients from body tissues, which can impair carcass yield as pointed out by Trocino *et al*. (43).

Transport

Under commercial conditions, rabbits are conducted at abattoir using commercial lorry which has two or three axles and a loading capacity ranging from 1,500 to 6,000 rabbits. The crates are placed on the vehicle in multi-floor crates roller stands. In an Italian recent survey Petracci *et al*., (28) found that the average transport time was 257 min (range: 20 to 800) corresponding to average distance of 203 km (range: 10 to 500). In a previous investigation conducted in Spain, Buil *et al*. (44) found similar journey durations and distance. Luzi *et al*. (45) conducted a survey in North Italy studying preslaughter transports from farms located at different distances (25, 50, 100 and 150 km) over one year. They observed that the most critical conditions for rabbits are when they are transported over 4 hours and at environmental temperature above 18-20°C and relative humidity of 70-75%. A close environmental control in the crates or modules on the vehicle is difficult, mainly because on most vehicles ventilation is passive and is impeded by the close stacking of adjacent crates. When lorries are full of rabbits, the ventilation inside tend to be poor, particularly when the truck stops, either during the journey to, or on arrival at abattoir so rabbits on the middle of crate stacks may suffer hyperthermia (1). The high probability of thermal stress being suffered by some rabbits in transit have been documented by De La Fuente *et al*. (38) who found that the rabbits transported in summer rather in winter showed signs of severe heat distress, since they had higher blood cortisol, lactate and glucose, creatine kinase and lactate dehydrogenase enzymes, and evidenced more dehydration, with greater osmolarity. Nevertheless the same authors observed that winter transport increased muscle activity as evidenced by lower liver and muscle glycogen concentration. Moreover Liste *et al*. (46) found that rabbits placed in the middle and bottom of crate stacks showed higher levels of some stress indicators (blood glucose, and corticosterone) than those located at the top floor without regard of journey length. A recent survey has evidenced that the prevalence of rabbit mortality between crating and hanging at the abattoir line is very low (0.079%) (28). As indicated before, mortality rate in rabbits is lower in respect with those found in broiler chicken, turkey and laying hens (24).

Conclusions

In the present review it has been discussed how welfare of avian species and rabbit is affected during transport in commercial conditions. Both in poultry and rabbits production chains, in main cases animals are transported twice during their productive life so welfare concerns are different in relation to considered category and age of the animals (breeders vs. slaughter animals, pre-deposition layers vs. spent hens; weaned rabbits vs. slaughter rabbits, etc.).

As regard for poultry species, major sources of welfare concern in transport of chicken broilers, turkey and laying hens are the process of catching and crating birds, environmental conditions in transit and journey with long duration. In accordance with the recent Regulation

2005/1/EC, to reduce these stressors, it is necessary to provide suitable feed and water the birds at least in the case of journey lasting more than 12 hours and vehicles must be equipped by devices able to maintain suitable environmental conditions (T and RH) to avoid extreme conditions (5-30°C).

Concerning rabbits, they seem to be more resistant than poultry to stress factors connected by transport phases as proved by the lower mortality rate and injury prevalence. Catching and crating seems to be the major critical points to impair welfare conditions during preslaughter. As for poultry, extreme environmental temperatures and relative humidity, feed and water deprivation and long journey can worse the welfare conditions.

It is also important to underline that animal welfare is becoming an ever increasingly important societal issue in EU. It is clear that the consumer quality definition of animal-origin food includes also views and perceptions about accordance of food production with good farming and preslaughter practices, with greater respect for the environment and for the welfare of animals. So that, the changing towards animal friendly and environmentally sustainable production systems can be allow to obtain added value products.

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ASPECTS OF ADAPTATION PHYSIOLOGY IN FARM ANIMALS

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Animal welfare has been historically dealt with by various schools of thought, which have accordingly set up different conceptual frameworks in their studies. Three major approaches can be thus recognized (1):
- The biological functioning school, which states that welfare is closely connected with the absence of a physiological stress response, or at least with the absence of a large stress response.
- The feelings school, which believes that welfare refers to what the animal feels; welfare would be thus characterised by the absence of "suffering", i.e. strong negative subjective states such as pain, fear and frustration.
- The animal behaviour and ethology school, which sets a correlation between animal welfare and possibility to perform the species-specific behavioural repertoire.

As a matter of fact, these approaches are not mutually exclusive and, often, a *balanced approach* is badly needed to draw reasonable inferences from complex issues which involve basic and applied science, moral obligations, social and cultural aspects of human communities.

Adaptation physiology studies adaptation and developmental conditions that influence adaptive capacity. I shall only deal in this paper with physiological parameters of adaptation to housing systems and metabolic demands in farm animals. For reasons of conceptual coherence, adaptation physiology has an obvious link to the above biological functioning school. This states (2) that the welfare of an individual is its state as regards its attempts to cope with its environment. Welfare can therefore vary between very poor and very good: it is a quantitative rather than a dichotomic parameter. Stress (2) is accordingly an environmental effect on an individual which overtaxes its control systems and reduces its fitness, or appears likely to do so. This concept implies that any possible stressing event should be offset against the animals' coping capacity. Various environmental effects may bear on welfare of farm animals; these can be grouped as follows:
- climate and microclimate conditions (temperature, humidity, draught, etc.);
- microbial infectious pressure;
- pain, fear, inability to perform a defined behavioural repertoire;
- barren environment, boredom;
- inadequate diet;
- metabolic stress for both milk and meat production;
- farmer's behaviour (stockman's effect).

The concept of adaptation can refer to three distinct levels (3): cell/organ, individual, population. Individuals are prompted to adapt in order to improve and optimize the interaction with their environment. In this respect, animals usually adopt a "feed forward" strategy: animals mount a corrective action to potentially noxious stimuli before whichever problem becomes substantial. This implies a stepwise corrective action, whereby activity and energy expense are proportional to the perceived threat. Animal needs bear on adaptation (3). Needs are meant as fundamental requirements in animal biology. Needs may refer to vital resources, or to particular

actions aimed at obtaining vital resources. Thus, for instance, pigs tend to get food by rooting in the soil and tend to handle forage as well.

On the basis of the above definition, welfare can be expressed as the result of the adaptation process, including adaptation to disease agents: in this case animal welfare is equal to animal health (3).

As for farm animals, welfare is much more affected by chronic rather than acute stress. Chronic stress may be in fact the long-term effect of modern husbandry techniques in intensive herds. These may in fact overtax the animals' coping ability and force them to long-lasting homeostatic control actions. Having in mind the 5 freedoms for farm animals (4), four of these may be more or less respected in intensive herds, whereas serious limits are almost invariably set to the animals' behavioural repertoire. Does successful adaptation to such conditions always imply good welfare? It is probably so in case of minor homeostatic adjustment. Adaptation by lengthy and/or complex, energetically costly homeostatic regulations are by no means associated to satisfactory welfare conditions, even though the clinical conditions of the individual are apparently normal. In fact, whenever animals are forced to severe, prolonged coping reactions with a considerable energy expense, welfare is poor and a serious depression of the immune system eventually turns out as one of the negative outcomes. The dramatic failure of potent Foot-and-Mouth Disease vaccines in Holstein cattle reared under hot climate conditions in Saudi Arabia is a very convincing demonstration of this tenet (5). Which rationale, which explanation in terms of phylogenetic evolution can be proposed for immunosuppression under stress conditions? It is worth dissecting this issue in models of acute versus chronic stress. Acute stressing events well beneath the host's threshold for coping are not dangerous and often conducive to useful learning experiences. Interestingly, transient acute stress may be even associated to a better immune response; such events may be thus thought of as nature's adjuvant under field conditions (6). Surely, this is not true of long-distance journeys of calves and pigs, which show in fact distinct signs of serious inflammatory reactions and immunosuppression, usually peaking at day 4-5 after transportation.

It can be argued that the high energy demand for coping under conditions of chronic stress forces animals to re-define their metabolic priorities to the detriment of the immune response. This is clearly shown in the leptin model: under starvation (chronic stress) leptin is shut off by adipocytes, which leads to serious defects of immune effector functions (7). Also, secondary antibody responses and immunological memory may be energetically costly (8) and therefore down-regulated during food restriction. In addition, a conflict often arises in farm animals between high levels of immune response and performance under conditions of continuous infectious pressure. The *M. hyopneumoniae* model in pigs is a very convincing example of this crucial link (9) A similar conflict can be envisaged as regards energy expense for milk production and immune effector functions in the early lactation period of high yield dairy cows.

In order to define animal welfare, physiological parameters of adaptation can be adopted together with the productive, pathological and behavioural ones. Which rationale can be defined for the use of physiological parameters? According to Moberg (10), stressful events are perceived as potential threats to homeostatic balance; this leads to the onset of a biological defense action, characterized by:

1. early, biological response (neuro-endocrine and behavioural);
2. later change of biological functions in different organs and apparata.

Immune effector functions are a very convenient readout of the changes referred to under point 2. Thus, good welfare conditions imply no or minor changes of fundamental immunological functions. Instead, notable, persistent changes indicate a complex, energetically costly adaptation process, which implies poor welfare even under seemingly good clinical conditions.

Immune functions represent a crucial reporter system of the adaptation process because of the strict functional and anatomical connections between brain and lymphoid organs; brain itself is the main regulatory organ of the immune system. The two main circuits, "Psycho-sensitive stimuli/behavioural response" and "Antigenic stimuli/immune response", are indeed subsystems of a unitary integrated complex aimed at providing optimal conditions for the host's survival and adaptation (Figure 1). In this conceptual framework, immune responses, stress and inflammation should be considered an ancestral, overlapping set of responses aimed at the neutralization of stimuli perturbing body homeostasis (11).
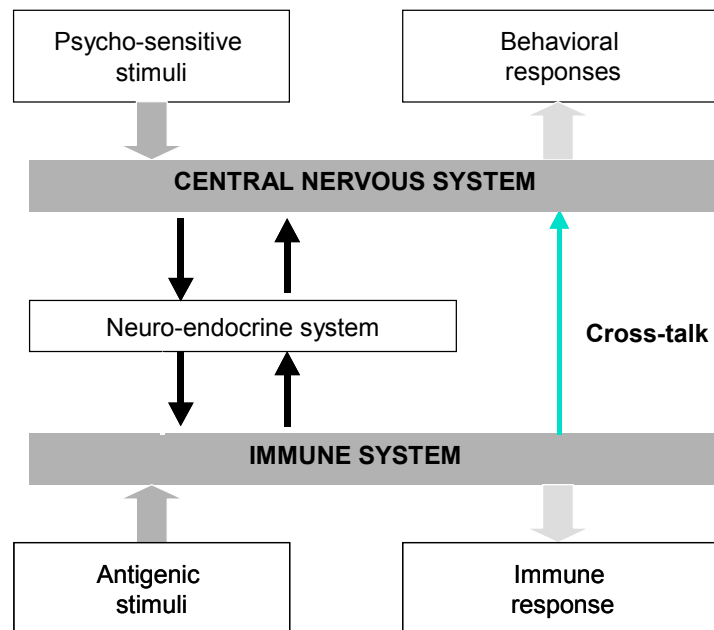
Figure 1. Cross-talk between nervous and immune systems

The effector mechanisms in the stress response are remarkably similar for both infectious and non–infectious stimuli, albeit differently modulated. Thus, a pro-inflammatory cytokine like IL-1 induces activation of the hypothalamo-pituitary-adrenocortical (HPA) axis as well as stimulation of cerebral noradrenaline; the effects of IL-1 are remarkably similar to those observed following either LPS administration (reminiscent of infectious stress) or acute, non-infectious stressing events in laboratory animals, such as electric shock or restraint (12). Likewise, the brain produces IFN-α in response to non-inflammatory as well as inflammatory stress; the intracerebral injection of this cytokine may alter the brain activity to exert a feedback effect on the immune system (13).

The crucial link between immune and behavioural responses is exemplified by the role of pro-inflammatory cytokines in the induction of sickness behaviour (letargy, anorexia, curtail of both social and reproductive activities), i.e. a clearly defined motivational status (14). The host adopts different behavioural priorities to mount a well-organized, integrated response to microbial infections; interestingly, depression is likely to provide an important adaptive

advantage to sick animals and anorexia is associated to a better chance for survival under such conditions.

A question obviously derives from the above statements: does high zootechnical performance always imply poor welfare in farm animals? The answer is not straightforward. On the one hand, high yields (meat and milk) require healthy animals, well adapted to their own environment; in this respect, poor adaptation is also associated to high culling rates. On the other hand however, high yields do imply high coping ability, which cannot be expressed by all members of an animal population; such risk is more serious in farms where an obvious discrepancy exists between animal genetics and farm management / logistics/infrastructure.

The relationship between welfare and production in farm animals can be depicted by a bell-shaped dose/response curve (15). Domestication has increased welfare of feral animals to a plateau level; welfare then diminishes or even reverses as animal production increases; a point of "acceptable" welfare should be roughly set in the middle of the descending part in the dose/response curve (15).

According to the above scheme, animal production should go along to a certain extent with the improvement of welfare conditions. Is there convincing evidence in the field? The answer is affirmative. On passing from individual crates to group housing, welfare and thriftiness of veal calves was on the increase as shown by daily mean weight gains, sanitary and laboratory parameters (16).

If immune functions represent a crucial reporter system of the adaptation process, it goes without saying that a sound panel of clinical immunology tests (complement, lysozime, serum bactericidal activity) may reveal a substantial decrease of the host's immune competence for environmental pathogens, which paves the way to opportunistic microbial infections (17). Interestingly, the above functions relate to the innate immune system, which does not have memory and acts irrespective of antigenic specificities by recognition of conserved microbial antigens. A defective innate immune response forces the host to a wider use of the adaptive immune response (antibody and cytotoxic T lymphocytes), which is by far more demanding in terms of energy expense. In turn, the efficiency of the adaptive immune response is also poor under conditions of chronic stress (5). Clinical immunological data should be interpreted with caution. Attention should not be focussed onto single, abnormal values. Instead, a comprehensive view should be adopted; its main parameters can be summarised as follows:

– the time kinetics of abnormal values;
– the prevalence of abnormal values in the group;
– the co-expression of different abnormalities in the same individuals in the herd:
– the association of some abnormalities to other ones with overt negative prognostic value (see e.g. leukocyte formula inversion + liver acute phase response).

Some models of response are briefly reported hereunder.

Acute stress: road transportation

With an obvious caveat with regard to seasonal and breed factors, long distance journeys of both cattle and pigs can cause one or more of the following effects:

– Sensory depression.
– Dehydration (higher Packed Cell Volume, total serum protein, haemoglobin).
– Signs of adrenal response (glucose, urea).
– Plasma PO4 increase and Fe decrease.
– Signs of cytokine responses (e.g. interferon-alpha) (18).

Chronic stress: pigs

Genetic selection for lean pigs has caused the appearance of some undesirable traits, which may worsen the adaptation process to modern husbandry techniques. Thus, the percentage weight of the heart muscle has decreased from 0.38% in wild boars to 0.21% in modern Landrace pigs (19). Such pigs also show an accentuated mean capillary-to-fibre distance in larger (type II) muscle fibres, which hampers an effective removal of toxic metabolites and favours lactic acid accumulation (20). In addition, the resulting tissue hypoxia induces conditions of persistent oxidative stress response, which paves the way to serious clinical conditions like Mulberry Heart Disease, Porcine Stress Syndrome, Osteochondrosis. Lean muscle pigs show in fact abnormally high serum concentrations of oxygen reactive metabolites (ROMs), as opposed to rural swine (19). Undoubtedly, the widespread appearance of devastating viral diseases in the 90ies (Porcine Respiratory and Reproductive Syndrome, Porcine Multisystemic Wasting Syndrome) is related to the above phenotypes of swine breeds. This concept can be adequately grasped having in mind that PRRS virus can increase the susceptibility of pigs to bacterial endotoxin (21) and that Porcine Circovirus 2 (related to PMWS) can down-regulate the homeostatic IFN-α response of pigs (22). The resulting cascade of inflammatory cytokine responses and/or its effects could be thus more serious in pigs with the above negative traits.

As a matter of fact, several opportunistic diseases of swine are preceded by distinct abnormalities of clinical immunological data. Among these, serum complement and, in particular, serum bactericidal activity are endued in our experience with a high prognostic value.

The cascade of inflammatory cytokine responses is actually submitted to complex control circuits, in which interferon-alpha plays an important role (23) both *in vivo* and *in vitro* (24). Such a homeostatic role can be also exerted by exogenous interferon-alpha, orally administered to pigs. The cytokine can be thus adopted for sound disease control strategies in problem herds (25).

Chronic stress: high-yield dairy cows

The dramatic increase of mean production volumes in dairy cows over the last 20-30 years has not been without costs. Over the same time period, there has been a notable decline in the reproductive performance, as shown by the data about "open days" (interval from calving to the next conception) and number of services per conception. As a result, longer lactations and an increase in the number of cows culled for reproductive reasons are often observed in dairy cattle herds.

In terms of adaptation physiology, high-yield dairy cows show chronic inflammatory conditions; these are of utmost importance in the transition period (pregnancy/lactation) and in the early phase of lactation when the usual negative energy balance reaches its plateau level. The most consistent signs are both negative (albumin) and positive (haptoglobin) acute phase responses, reduced liver functions (bilirubin, vitamin A) (26), monocytosis, persistent inversion of leukocyte formula and albumin/globulin ratio, higher alpha-globulin concentrations, lower levels of haemolytic complement (alternative pathway) (27).

The above findings show that dairy cows must adopt complex adaptation strategies to cope with their metabolic stress. If adaptation is not successful, overt disease may ensue (mastitis, endometritis, clinical chetosis), which paves the way to early culling. Two distinct negative prognostic parameters can be recognized:

− A persistent acute phase response (haptoglobin) in the 2nd week after calving.
− Reduced dry matter uptake in the last days of pregnancy, which significantly bears on post partum complications (28).

Anorexia is thus a crucial event, which may dictate the subsequent fate of dairy cows. It undoubtedly deserves further studies to fully understand its patho-physiological basis. In particular, the correlation between anorexia and inflammatory cytokine response in the transition period should be further investigated in the next future.

Conclusions

Adaptation physiology reveals the complex adaptation strategies of farm animals under modern husbandry conditions. As such, adaptation physiology can show the extent and the duration of the adaptation process, as well as its complexity. At the same time, it provides useful parameters for sound animal welfare evaluation and disease control at the herd level. Last but not least, proper records of selected physiological parameters could be adopted for quality assurance purposes, aimed at a better qualification of food producing chains.

Acknowledgements

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HUMAN-ANIMAL RELATIONSHIP IN AN EVOLUTIONARY PERSPECTIVE

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The meaning and evolution of the history of human – animal interaction finds its roots into the natural biology of all the living organisms. This interaction evolved through a series of steps. Most likely, at the beginning human beings started to exploit animals catching and eating them, as the predators with the prey (1). Then, gradually, humans realised that some animals, instead of being prey themselves, could have been useful helpers in hunting other preys. On the contrary, other species could be kept in order to obtain meat and other products. Thus an alliance started to be established, giving potential benefits both to humans and animals, but giving man also the task to take the responsibility of caring for the animals. This collaboration passed through different phases, from taming to domestication. According to Price (2), domestication may be defined as "the process by which captive animals adapt to man and the environment he provides. Adaptation to the captive environment is achieved through genetic changes occurring over generations and environmental stimulation and experiences during an animal's lifetime".

A number of reasons may have lead to keep animals in captivity and to domesticate them through genetic selection. The main reasons may have been to obtain animal products, as well companionship and also the possibility to carry out scientific research (1).

The domestication process involved species which had peculiar traits allowing these species to be domesticated. The main characteristics may be summed up in the following ones: the possibility to reduce the flight distance towards human beings, the sociality, the mating and feeding strategies. According to Galton, recognized as the "father of behavioral genetics", six conditions, related to some behavioural and physiological attributes, facilitated the domestication process. These conditions may be summarised as follows: animals should be 'hardy', i.e. the young animal has to be able 'to survive removal from its mother' adapting to new environmental conditions; they 'should have an inborn liking for man' and also be social animals; 'they should be comfort-loving', accepting to stay in a pen or closely together; 'they should be found useful for savages', i.e. to be an 'easily-maintained source of food'; 'they should breed freely', without the need to maintain breed colonies; 'they should be easy to tend', i.e. their reactive trends have to be easy to control in order to allow them to be kept together.

As far as farm animals are concerned, among the first domesticated species were goats, sheep, cattle and horses, followed by pigs and more recently rabbits (3).

The changing of human lifestyle progressively induced modifications in the husbandry and management systems, from rural to more intensive ones, till reaching the industrial husbandry systems widely spread up to date. These last systems may pose serious welfare problems to the adaptive capabilities of animals, thus lowering their quality of life, i.e. their welfare levels. This problems stimulated the research on the possibility of defining and quantifying farm animals' welfare (4, 5). In fact, in rearing animals, humans should take into account the following so called 'five freedoms' (6, 7):

- *from hunger and thirst*
  by ready access to fresh water and a diet to maintain full health and vigour;

– *from pain, injury and disease*
  by prevention or rapid diagnosis and treatment;

– *from discomfort*
  by providing an appropriate environment including shelter and a comfortable resting area;

– *from fear and distress*
  by providing conditions and care which avoid mental suffering;

– *to express normal behaviour*
  by providing sufficient space, proper facilities and company of the animal's own kind.

The science of animal welfare is a multi-faceted issue and implies scientific, ethical, economic and political dimensions (8). Thus this science needs an interdisciplinary approach, bringing together researchers from different disciplines within the biological sciences, such as physiology, veterinary science and ethology.

The interdisciplinary approach, combining ethology, physiology and psychology and the studies of the human-animal interaction, may give the advantages of improving the understanding of knowledge about animal welfare issues as well as to obtain methodological gains. The collaboration among ethologists, physiologists and psychologists has produced a model to interpret stockperson-animal interactions (9).

In order to assess whether there are welfare problems for the reared animals, it is necessary to find out scientific objective welfare indicators which can be measured directly on animals. The fundamental importance of carrying out scientific research has to be stressed, in order to avoid misunderstandings due to rather extreme positions, both from mechanistic and welfarist viewpoint, and avoiding also anthropomorphism.

Welfare indicators include behavioural, physiological, health and production variables (10, 11). Behaviour may be considered as the first and most evident sign of the organism's status. Abnormal behaviours are behavioural indicators of stress and may seriously impair animals' welfare (12).

Besides the ethogram (i.e. behavioural repertoire), also the response to behavioural tests is useful in evaluating animal welfare, for example in order to study the 'temperament', i.e. the behavioural reactions which may be related to fear and anxiety. To evaluate the effect of different husbandry systems on the animal reactions, tests aimed at measuring fear in a new environment or towards humans may be used: for example 'open-field' (13, 14); 'emergence' and 'tonic immobility' test (15).

Welfare indicators have to be studied according to the species characteristics and evolution. In this respect, domesticated species differ in some way from their wild ancestors. For example some behaviours basically differ form wild to domestic animals: the last ones have lower flight distance from humans, and are able to reproduce themselves in large quantities also without courtship behaviour, being in many cases artificially inseminated. These differences are related to behavioural and physiological changes induced by the domestication process.

However, in spite of their adaptive capabilities, also domestic animals may suffer from distress: when the environmental stressors dramatically threaten their possibility to cope, they may show many behavioural and physiological alterations. Finally, also their health status may be compromised, possibly due to reduced immune resistance as a consequence of distress (16). Stress induced reactions in animals include behavioural and physiological modifications aiming at *coping* towards the stressor. The *coping* strategies depend from many factors, such as the species, the individual genetic trends and experience (17, 4).

The biological available responses include behaviour as well the activation of the autonomic, neuro-endocrine and immune systems, which interact and allow the organism not to be overcome by pathogenic challenges (16, 18).

Provided that husbandry and management systems are carried out according to the species' needs, farm animals may adapt rather well to the rearing environment, thus maintaining rather high welfare levels and reaching also good productive performances. This may represent a further step in the collaboration, between animals and man, looking at the evolution of the domestication process and at the human-animals interaction as a biological process. Of course this means that man has not only to rear animals, but more and more to care for them, improving their quality of life and avoiding undue distress. The great amount of research which have been carried out and the process of going on dealing with farm animals' welfare aims at evaluating the effects of the different rearing systems on animals and to improve the rearing systems themselves. The direct effects of human – animal interaction have been now also widely investigated, showing, for example in swine, that positive interactions may improve welfare as well as production (9). Handling by humans in early life may reduce animals' fear toward human beings. This has been shown for example in rabbits (11, 19, 20).

As far as companion animals are concerned, the human-animal interaction has greatly changed in the last decades. The dog has a very old history of domestication, and the domestication process has lead also for dogs, like for the farmed species, to the selection of many different breeds according to their morphology and attitudes (21). Clutton-Brock (3) underlines the importance of the dog's domestication like a cultural event in the history of man. The dog is probably the first domestic animal (22) and it has an in-built ability to understand human postural language. In fact, it seems that during the domestication process man has selected some social-cognitive skills that enable dogs to communicate with man in a preferential way (23). Thanks to this kind of social learning, dogs have been fast admitted in human world. Nowadays, there are many breeds of dogs, with a morphological variety and consequently there are many activities in which man and dog cooperate all together.

One of the new frontiers of the human-pet relationship is the employment of animals in therapeutics activities. The effects of pets on human health date back many years ago and some researches have tried to investigate, in a scientific way, the positive implications of this relationship (24, 25). The involvement of animals in social care institutions has a long history, even though the labels and means to conduct these programmes have varied widely. Although animals were not the primary focus of this early therapeutic approach, animals did constitute an essential component of the treatment (26).

Nowadays, pets are often an integral part of human family unit and this aspect has many social and emotional implications. Pets generally spend all the lifetime together with humans, as members of the family, adapting to the life style of the owner and to the home environment. In spite of the fact that human – pet interaction may be very successfully, giving both a lot of benefits, sometimes behaviour problems may appear. In fact also pets, in spite of their close bond with humans, or possibly sometimes due to this latter, may suffer from stressors related to their living environment, including owners' behaviours and attitudes towards them (27, 28). The onset of some pets' behavioural problems (for example: aggression and anxiety related disorders) may modify the relationship with the owner and have dramatic consequences for the animal welfare, till even euthanasia or abandonment to a rescue shelter (29). Thus it's very important to assess also pets' welfare by means of physiological and behavioural indicators, in order to detect every conditions of acute or chronic stress that can alter animals' physical or psychological health.

We can consider disturbed behaviours, excluding the ones related to organic and/or neurological diseases, as:

1) apparently 'anomalous' reactions which actually are included in the behavioural repertoire of the species and whose aetiology is explained in the context of appearance. However these behaviour may be very disturbing for the owner;

2) behaviours induced by environmental stressors, or derived by genetics predisposition, such as the reactive trends and threshold. These behaviours may have an individual component, but generally are raised by multiple causal factors, at the individual and environmental level.

Disturbed behaviours in pets have generally a multi-factorial aetiology, including individual and environmental variables. Up to now the research is mainly focusing on the possible individual genetic predisposing factors and the relationship among disturbed behaviours, mainly the anxiety related ones, and some neurochemical modifications which involve noradrenergic, dopaminergic and serotoninergic neurotrasmission systems (30, 31). Of course the individual experience, i.e. learning, and the environmental effects, mainly the owners' attitudes, have however to be considered as important factors which may raise and/ or facilitate the onset of behavioural problems in pets.

The improved knowledge of pets' needs may help in avoiding, reducing and/or eliminating also disturbed behaviours. Veterinary behavioural medicine has developed and is now growing more and more in order afford these problems (32, 27, 28).

In conclusion, human-animal relationship has to be considered in an evolutionary perspective, both for farm animals and for pets. Man has started to discover how the animals he domesticated live and feel. The possibility to improve this interaction in a collaborative way is related to deepen this knowledge and to care for animals according to the results of the scientific research.

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HUMANS-DOGS (AND DOGS-HUMANS) RELATIONSHIP: EMERGING PROBLEMS IN URBAN AREAS

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The origins of the domestic dog

The dog family or Canidae is a biologically cohesive group of carnivores that is divided into 38 species, including the domestic dog, *Canis familiaris* (1). Although the members of this family share a number of ethological features (especially those related to social behaviour), the dog was the only *Canidae* species (and also the first animal specie) to be fully domesticated by humankind.

Since before Charles Darwin, the hypothesis on the origin of the domestic dog from the wolf (Canis lupus) or the golden jackal (Canis aureus) elicited a fervent debate. In the 1868s, with regard to the origins of the numerous domesticated varieties of the dog from a single wild species or from several species, Darwin wrote: "We shall probably never be able to ascertain their origin with certainty" (2). A long time later, Konrad Lorenz popularized the idea that some modern breeds of dog were originated from the wolf, but the others instead were derived from the jackal (3). However, afterwards Lorenz invalidated this hypothesis, especially on the basis of the complexity of the jackal's howling repertoire, which is quite unlike to that of the dog or wolf (4). Recent results, deriving from a combination of studies of ethology, morphology and molecular genetic, indicate that the principal, if not the only, ancestor of the domestic dog is the wolf (1).

Humans perhaps domesticated the wild dog ancestor more than once, and domestication was an event that took place independently in different geographical areas. Indeed, from as early as the Middle Pleistocene period, the bones of wolves have been found in association with those of early hominids in different archaeological sites dated between 400,000 and 150,000 years BP (Before Present; see 1). Examples include the site of Zhoukoudian in North China (5), the cave of Lazeret near Nice in the south of France (6), and the site of Boxgrove in Kent, England (1). These archaeological discoveries demonstrate that the sites of occupation and hunting activities of humans and wolves must often have overlapped.

However, the first evidence of dog domestication come from archaeological records that date back to about 14,000 years ago, during the cultural period known as the Epipaleolithic or Natufian. In particular, of special value has been the discovery in the Natufian site of Ein Mallaha in Israel (12,000 years BP), of the burial of an elderly human with a puppy (a tamed wolf or "dog") of about 4-5 months of age. The human skeleton lay in a flexed position, with its hand on the thorax of the puppy (7).

It is likely that dog domestication corresponded to a change in hunting strategy by a few human populations. Indeed, during the Natufian, humans developed the practice of the long-distance hunting by using new tools such as the microliths (arrows armed with tiny stone blades). The success of these long-distance projectiles would have been enhanced by the new partnership with dog, which could help to track down, immobilize and carry back wounded animals. Such a cooperative hunting technique would thus have resulted in greater hunting efficiency (8).

Undoubtedly, the dog domestication represented an advantage to humans' evolution. Indeed, once tamed, dog became not only an efficient partner in hunting practice, but also an ideal guardian and an incomparable companion "pet" animal, being able to elicit attachment and engaging in complex communicative interaction with its human partner.

The human-dog relationship

People always describe the human-dog relationship as a partnership, a dyadic symbiosis, a reciprocal special bond sealed by a cross-specific imprinting, or as the result of a combination of both biological and zooanthropological factors. Certainly, domestication has enriched the dog natural behavioural repertoire, providing this animal with new behavioural modules (or, more likely, a selection process magnifying pre-existent behavioural items), which, in turn, have facilitated the communicative interactions with the human beings. Thus, domestication might have reinforced the reciprocal emphatic bond in the dyad human-dog by enhancing the dyad skill known as the "reciprocal mind reading", namely the reciprocal ability to read and modify emotions without sharing an articulate language.

Recent evidence from ethological studies confirms that the domestic dogs are unusually skilled at reading human social and communicative behaviour – even more so than both wolves who were raised by humans and chimpanzees who are more closely related to human phylogenetically (9-12). For example, they use human social and communicative behaviour (e.g. a pointing gesture or gaze cues) to find hidden food, and they know what the human can and cannot see in various situations (9, 10). These social-communicative skills seem to be a dog specie-specific feature, and represent a case of convergent evolution with humans, manly due to adaptation to human forms of cooperation and communication. Comparative studies between canid species, and in particular studies on foxes, *Vulpes vulpes*, experimentally domesticated, suggest that these unusual human-like social skills have a heritable component and initially evolved during domestication as a result of selection on systems mediating fear and aggression towards humans (10, 13-19).

Changes in the human-dog relationship and emerging problems in the urban areas: the case of the "dangerous dogs"

Since its domestication the dog has mainly had a functional role within the human rural communities, being used in hunting practices and to protect the human family, the house or the livestock. However, the socio-economical progress and urbanization of the Italian population following the end of the Second World War have changed the traditional human-dog (and dog-human) relationship, leading to management problems. Indeed, keeping dogs of any kind of breed or size as pets has become increasingly popular. As reported by the Italian Ministry of Health (Ministero della Salute), Italian families kept about 5.35 million of dogs as pets between 2005 and 2006 (20). However, the urban domestic environments are quite different from the rural environments, where these animals can more easily develop and maintain a cognitive and affective independence. Furthermore, some people have an overwhelming tendency to "personify" dogs, limiting or actively repressing the expression of their natural behavioural repertoire.

Adopting dog as pet requires to the human family the ability to face management problems, which, when not adequately managed, might eventually change the relationship with this pet, compromising its psycho-physical welfare while increasing the risk for regrettable incidents mainly related to excessive aggressive reactions. In reference to this, the case of the "dangerous

dogs" represents an issue of topical interest and very often an example of inadequate management of the dogs by their owners.

A study performed by the Department of Health and Human Service of the Centers for Disease Control and Prevention (USA; 21), reports that in 2001, an estimated 368,245 persons were treated for dog bite-related injuries in the United States (rate: 129.3 per 100,000 population). The injury rate was highest for children aged 5-9 years and decreased with increasing age. Approximately 154,625 (42.0%) dog bites occurred among children aged ≤14 years; the rate was significantly higher for boys (293.2 per 100,000 population) than for girls (216.7). For persons aged ≥15 years, the difference between the rate for males (102.9) and females (88.0) was not statistically significant. The number of cases increased slightly during April-September, with a peak in July (11.1%). For injured persons of all ages, approximately 16,526 (4.5%) dog bite injuries were work-related (e.g., occurred to persons who were delivering mail, packages, or food; working at an animal clinic or shelter; or doing home repair work or installations). For person aged ≥16 years, approximately 16,476 (7.9%) dog bite injuries were work-related (21).

Data collected by searching for news accounts and by use of The Humane Society of the United States' registry databank indicate that from 1979 through 1998, dog attacks resulted in more than 300 human dog bite-related fatalities (DBRF) in the United States (22). Most victims were children. At least 25 breeds of dogs have been involved in 238 human DBRF reported during this 20-year period. Pitt bull-type dogs and Rottweilers have been responsible for 66 and 39 human deceases respectively (22). However, although fatal attacks to humans appear to be a breed-specific problem, other breeds may bite and dangerously injure persons at higher rates. Indeed, fatal aggressions represent a small proportion of dog bite injuries to human, constituting less than 0.00001% of all dog bites annually (22).

With regard to Italy, up until today, very few and scattered studies have been focused on dog bite attacks towards humans and DBRF. Data mainly from non-official sources, such as newspapers and reports published on the web, indicate an estimation of 400-700 cases per year of persons incurring in nonfatal dog bite-related injuries, and 1-2 cases of deaths related to dog attacks per year. Most victims are children and elderly. However, cases of human dog bite-related injuries are most probably underestimated. Indeed, bite related incidents often are not denounced to authorities, injured persons and dogs' owners deciding amicably to negotiate the event, especially if bites do not result in serious injuries.

Episodes of canine aggressions on persons occurring in the last years - often also markedly pointed out by mass media - have become a serious problem of epidemic proportion, engaging on the front line the Italian Minister of Health to control this public health concern. In particular, the question on breeds of dogs that might result "dangerous" to humans has been a topic fervently debated by public opinion, and on September 2003 the Minister of Health has enacted breed-specific restrictions by an ordinance, listing 93 breeds of dogs as potentially "dangerous" to public heath (23). However, on October 17th 2003, the Consiglio Superiore della Sanità has rejected the list of "dangerous" dogs, indicating that "dangerous" breeds *per se* do not exist. As a consequence, the list of "dangerous" dogs has been modified and initially restricted to 18 breeds (24), and, more recently, further shortened to 17 breeds, Neapolitan mastiff being cancelled from the official list (25).

Clearly, genetic history can influence aggressiveness of breeds and individual dogs, selective breeding increasing or decreasing these tendencies. However, it is very important to keep in mind that levels of dog aggressiveness are mostly influenced by several environmental variables, including the extent of dog socialization to people (especially children), its training, the quality of its psycho-physical welfare, and the owner's ability to prevent and manage contexts that might induce dog excessive aggressive reactions. Thus, an increased knowledge of

dog's ethology can help people to avoid unpleasant incidents and improve the quality of the relationship with this pet.

Dog communicative signals: the language of body postures and facial expressions in social interactions

Notwithstanding the process of domestication, most of the ethological features related to dog social behaviour have not changed, and therefore they closely resemble those characterising the dog wild ancestor, the wolf.

Wild wolves are carnivores living in packs. The pack usually consists of the *alpha* individuals (the breeding pair) and their offspring. The *alpha* individuals actively try to suppress mating in the rest of the pack by agonistic behaviour (26,27). Two parallel hierarchies can be detected in the pack, a male and a female one. Both are essentially pyramidal in structure, since rank differences are most obvious between high-ranking individuals, and are less distinct between middle-ranking adults and between pups. There is generally a close relationship between age and rank, the oldest animals occupying the top of the hierarchy. Cross-sex dominance relationships between males and females of similar rank are weak or non-existent. The *alpha* female is highly aggressive towards other females in her pack before and during the mating season, apparently in order to prevent them from breeding. The *alpha* male tends to be highly aggressive towards intruders, but not to other pack members. A *beta* male can sometimes be distinguished, and an individual with this rank will often be the most aggressive male in the pack, but will reserve aggression towards the *alpha* male for direct challenges to his leadership. Low-ranking wolves tend to be sociable both inside and outside the pack (26,27).

The domestic dog refers to its human family in a way resembling that of the wolf pack. Indeed, a dog identifies as its pack the human family (by which it has been "accepted"), and recognizes a human member of the family as the *alpha* individual. Moreover, during its social interactions with both conspecifics and non-conspecifics, dogs use communicative signals similar to those used by wild wolves to communicate to each other, namely body postures and facial expressions (3,28,29). For example, both dog and wolf communicate their dominant social status by an upright body posture with the head and tail held high and the ears pricked. An aggressive dominant animal couples this body posture with raised hackles, curled lips and bared teeth. In contrast, subordinate dogs or wolves hold their body low, the ears flat, and the tail held low and close to the body, creating the general impression of a smaller animal. Subordinates that display a fearful behaviour exaggerate these postures by cringing, tucking their tails between their legs and generally reducing the overall apparent body size. Subordinates often approach dominant individuals in an enthusiastic greeting with extreme wagging of the tail whilst maintaining a low general body posture (30). This behavioural pattern may also be associated with nuzzling and licking the face of the dominant animal; such behavioural pattern has been also observed among wolf pups directed towards other members of the pack and among dog pups directed towards their mother, in both cases facilitating/encouraging food regurgitation by the adult. Subordinate displaying the posture for passive submission rolls on its back exposing its inguinal region (submissive urination may also occur), a behaviour evoking pup posture when its mother rolls it on its back and licks its ano-genital region to stimulate pup urination and defecation. Dogs show a similar behavioural pattern also in the interaction with their human family, performing an "enthusiastic" greeting ritual to a family member returning home after a period of absence.

Also dog communicative signals associated to play solicitation behaviour are similar to those observed between young wolves. The more common signals, especially in dogs, include the play bow, pawing with a front foot, twisting jumps and open mouthed panting (31).

Unlike wolves, dogs use a complex tail-wagging repertoire to communicate a variety of moods related to a variety of contexts (30). Loose, free tail-wagging indicates general friendliness, and often extends to incorporate the entire rump in subordinate animals. More anxious or nervous dogs tend to wag their drooping tails more stiffly, seemingly as a pacification signal. Rapid, stiff, upright "flagging" of the tail indicates threat and possibility of aggression (30).

Recent ethological observations have demonstrated that dogs perform asymmetric tail-wagging behaviours in response to different emotive stimuli (32). In particular, stimuli that could be expected to elicit approach tendencies, such as seeing a dog's owner, are associated with higher amplitude of tail-wagging movements to the right side, while, stimuli that could be expected to elicit withdrawal tendencies, such as seeing a dominant unfamiliar dog, are associated with higher amplitude of tail-wagging movements to the left side (32).

Dog aggressive behaviour

As already explained in this paper, aggression is the most commonly reported category of behavioural problems in domestic dogs. However, it is important to keep in mind that both intra- and inter-specific aggressive behaviour are a natural feature of both wild canids and domestic dogs behavioural repertoire. In addition, under natural conditions, the aggression of wild canids is held in check by the set of body postures and facial expressions previously described in this paper, which clearly communicate to the opponent animal the aggression motivation or intent. Usually, aggressive encounters are rapidly ended when one individual displays the "cut-off" behaviour, such as submissive postures and infantile vocalizations (whining, yelping; 33,34). Biting is a key component of the predatory behaviour in canids (predatory aggression; see Table 1). However, there are other contexts in which a dog, if not adequately trained by its owner, might display aggression by attacking and biting persons. Table 1 reports a schematic description of dog aggression contexts/circumstances.

Table 1. Contexts and circumstances of dog aggressions

Aggression	Context/Circumstances
Competitive	Attempt to acquire hierarchical privileges: control of space, sleeping area (bed, furniture, place on the carpet, etc.), social or sexual partners
Irritation	Frustration, hunger, pain
Maternal	Defence of the offspring
Fear	Inescapable and dangerous situations
Predatory	Hunting and capture of prey
Territorial	Attempt to avoid intrusion of invaders into (a part of) the pack territory

For example, dogs showing "competitive aggression" (also known as "dominance aggression" or "dominance-related aggression") tend to react aggressively to apparent challenges to their positions within the social hierarchy. These circumstances include those in which the owner is apparently treated as a competitor for resource (e.g. food, space, sleeping position, etc.) or in response to supposedly "dominant" gestures by the owner, such as holding,

petting, grooming, restraining, punishing or pushing past the animal, staring or yelling at it, or even leaning over it (35).

Commonly, competitive aggression is characterised by threats or attacks directed at the owner or a member of its human family rather than strangers. This form of aggression is also more commonly reported in intact males and neutered females, and it is one of the most frequent problems described by behaviour therapists and trainers (36-39).

Extreme "territorial aggression" represents another common behavioural problem in dogs. Like wolves, many dogs, especially those of guarding breeds, display a natural tendency to react aggressively to unfamiliar intruders (people and animals both conspecifics and non-conspecifics) within their home ranges. Home range or territory usually includes the immediate vicinity of the owner's home, but may also include other areas where the dog is regularly walked or confined. However, training the dog to bark without attack and bite an intruder invading the territory could prevent behavioural problem related to extreme territorial aggression. In reference to this, it had better train the animal to "control" its aggression towards intruders ever since its puppyhood.

Finally, social isolation (especially at puppyhood), restraint (such as chaining and the constriction in restricted spaces limiting dog performance of kinetic activities), and, more in general, all those life conditions preventing the expression of the dog natural behavioural repertoire, might lead it to experience fear, boredom, deprivation and frustration, with consequent excessive aggressive reactions towards people.

As previously reported, levels of aggressiveness may be affected by genetic factors. However, increasing evidence from ethological studies indicate that both social environment and events experienced during particular periods of the dog development can have more important effects than genetic influence on levels of aggressiveness at adulthood.

The development of dog social behaviour

A variety of studies on both human and animal models indicate that early social events, experienced during the so-called "critical periods" of the development, can induce long-term effects on social behaviour, predisposing to behavioural disturbances at adulthood.

As shown in Figure 1, among domestic dog pups, primary socialization period runs from about the third to the twelfth week after bird, with a peak of sensitivity between 6 and 8 weeks (35,40).
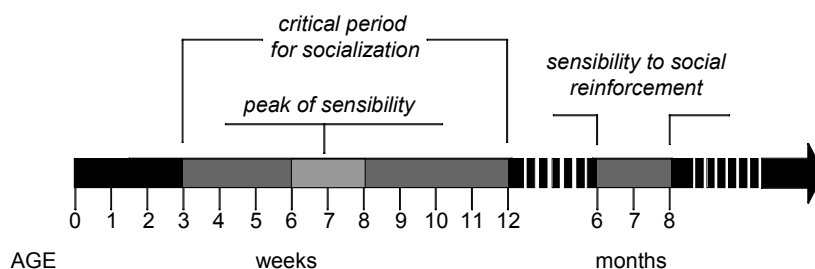
Figure 1. Critical developmental period for dog socialization ranges between 3 and 12 weeks of life, with an optimum between 6 and 8 weeks, and a period of sensibility to social reinforcement ranging between 6 and 8 months of life. Early social experiences, periodically repeated until about 8 months of age, favour attachment relationships with both conspecific and non-conspecific, determining the young dog's future social partners

Indeed, below 3 weeks of age, puppies' central nervous system seems too immature to permit socialization, and beyond 12 weeks their growing tendency to react fearfully to novel persons or situations could represent a limit for further socialization. However, between 6 and 8 weeks pup's social motivation to approach and make contact with unfamiliar persons or animals prevails over its natural diffidence towards novelty. Thus, this period represents the optimum time for socialization (35,40).

Among wild wolf pups, primary socialization ensures that the young animals consolidate social attachment relationship with their littermates, parents, and the other pack members. For what concerns the domestic dog, pups exposure to an adequate socialization during the developmental sensitive period allows they to form attachment relationship not only with conspecifics, but also with those non-conspecifics encountered in this period (41). Indeed, cross-fostering experiments demonstrate that dog puppies raised throughout the socialization period with only kitten littermates, as adults show a marked tendency to engage social interactions with cats and kittens, while the they tend to avoid conspecifics (42). Thus, the feature of the socialization experience not only determines the young animal's future social partners but also defines the species to which it effectively belongs. In addition, during the sensitive period for socialization, puppies also form the so-called "site attachment", namely the attachment for particular places. Therefore, during this developmental period, both the social and physical environment seem play a crucial role in determining pups attachments to both the living and non-living components of their rearing environment (40).

Ethological studies indicate that dogs reared in socially and physically impoverished environments from weaning until around 12-14 weeks of age as adults tend to exhibit neophobia when placed in unfamiliar situations, showing fearful and avoidant responses to anything novel or unfamiliar (35,43). However, in the absence of periodic social reinforcement until the age of 6-8 months, both young wolves and dogs, which were well socialized at 3 months, could, nevertheless, regress and become fearful again (see Figure 1).

Therefore, experiences, especially during the socialization period and later, between 6-8 months of age, plays a major part in determining which fears are acquired and how strongly they are expressed in adult life (see also 44). As a consequence, the provision of an enriched social, and stimulating physical, environment, by both breeders and owners, during the first 8 months of dog's life, may reduce the incidence of aggression-related behavioural problems, improving therefore both dog welfare and the quality of the dog/human relationship.

Conclusions

Among the different species of companion animals, the dog is the most preferred and desired pet by people, especially children. Certainly, many behavioural patterns displayed by dogs elicit a special attachment in the human partner. However, life together a dog requires diligence and a sense of responsibility from the human family.

Before adopting a dog, it should be opportune to consult a professional (e.g. veterinarian, dog behaviourist, or responsible breeder) to choose a suitable dog breed, sex, age, and temperament on the basis of the family's lifestyle and both the physical and social environment where the dog will live. Moreover, family members should spent time with dog before adopting it, especially in the case of families with children. In reference to this, it is advisable for parents to be sensitive to cues that a child is fearful or apprehensive about a dog: in this case it is important to delay the adoption of a dog. However, parents never should leave infants or young children alone with any dog. Children, in turn, should be educated for basic safety around dog.

Aggressive behaviour is a natural component of the dog's behavioural repertoire, which could become dangerous for persons because owners are often unable to adequately train their animals, or they are unable to prevent and/or manage those contexts eliciting responses excessively aggressive by their dogs. Often, owner's management mistakes are related to a poor knowledge of dog's ethology. Therefore, owner's education represents the only strategy to reducing unpleasant incidents due to canine aggressions. Indeed, an increased knowledge of dog's ethology can improve its psycho-physical welfare, reducing the misunderstanding risks with its human partner.

Acknowledgments

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CONTRADICTION IN HUMAN RELATIONSHIP WITH PETS

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Animals in Western society are taken under growing consideration.

The legal status of "things" is widely considered not sufficient to acknowledge the adequate respect that is felt necessary from a moral point of view.

In regard to animals in zootechnical productions, the European Union and in minor measure, but initial, the World Trade Organization are requiring in growing measure the definition and respect of the set of rules relating to animal welfare. This being no more just a simple expression of psychological condition, but has now become a precise scientific discipline.

In regard to pets the attention to the quality of living conditions is constant both by owners, who are ready to spend whatever is required for the welfare of their animals, for the therapy expenses in case of illness and for every day needs, and by political authorities who promote rules for the protection of animal interests.

In this situation apparently idyllic for the animal world, it is possible to find a few contradictions that can undermine and upset the practical result of a noble and shared purpose.

Animals cannot, for obvious reasons, directly express their interests or wishes, nor by means of a lobby action can they negotiate a legislation in their own favour.

On the other hand, it is not so sure that those amongst the human beings who worry the most about the conditions of animals are really able to always recognize a presumable and unexpressed animal interest.

The production of goods and services for animals, even it were really directed to satisfy their needs and not those, more or less conscious of their owners, would have difficulty in finding the best target.

Much more realistically, we must think that when we try to improve the condition of animal life in our society which is convinced about the achievement of this goal, we cannot help asking ourselves if we are really doing the right thing or whether on the contrary, we are only doing something that eliminates our collective sense of guilt that leads us to another consumer frontier.

Caring for the interests of animals, for their welfare or even for their rights, must not probably be the major social and political concern of our time. But if we do care, but then we achieve results we had not hoped for or that are even opposite to what we had expected would be very bad and should raise general indignation.

It is therefore an urgency not to run this risk.

The reason why it is easy to make this mistake is essentially based on the frequent banal way in which animals and their existences are treated, both when considered as previous phase to food or clothing, and when considered as companions in our lives.

The superficiality, that is to say, the not careful deepening of the matter, can be tackled by means of informed, multidisciplinary, plural values debate which is typical of Bioethics.

The acknowledgement, even in animal matters (that are often those of the humans that live with their animals),of dignity for complex problems that concern ethical considerations in

relation to the development of Biological, Zootechnical and Biomedical Sciences, can be, apart from a good will action, also a real obstacle to superficiality.

The awareness of the theoretical and practical difficulties and the provision of the right instruments represent the best deterrents against the risk of ending up with the output of different or even opposed results compared to the noble purposes that we wanted to pursue.

Social and economical data

In a recent opinion poll carried out in Italy (28/8/06 by Istituto Piepoli for the Festival of Philosophy 2006), the result was that half of the pollees believed that animals have a soul like humans.

Furthermore, according to 95% of the pollees, pets have rights, and according to 67% of the pollees, these rights are attributable also to non domestic animals.

Such opinions were somehow expected, but the dimensions of the line-up in favour of the animals by almost half of the pollees, cannot leave us cold.

Besides, the fact that almost half of the pollees recognize a spiritual element like the soul in animals indicates how, beyond a juridical kind of need to satisfy the request for rights, there is a great moral principle sincerely and widely spread that aims to elevate the status of animals.

The reason why somehow a greater importance is given to pets must be taken into consideration and it gives the biological phenomenon of domestication a significant social meaning which must be carefully studied in its origins and in its effects.

According to Eurobarometer data (8th June 2005), the majority of the European Union citizens thinks that the well-being programme for animal productions does not receive enough attention in agricultural policies. Moreover, over half of the pollees in the 25 member Countries (55%) thinks that the European Union is not doing enough to ensure a good condition of animal welfare.

This fact seems to fully justify the European policy in regard to animal welfare in zootechnical productions and the European request to consider animal welfare as a significant element in controlling world commercial exchanges.

The Council of Rome, through its animal rights office, has carried out a research on the economical and occupational importance of dog connected issues.

The results show that the progressive increase of pets and the progressive improvement of their standard of living equals a rise in expenses; among these, it is significant to notice how 11.5% consists in the purchase of accessories, undoubtedly less indispensable elements compared to food and veterinary treatments and therefore such to allege some sort of consumism even in regard to purchases for animals.

The fact of spending amounts between 500 and 1000 Euros to buy a certain breed of animal raises the doubt that, even in this case, there is a consumistic side to it, in fact, often the breed is not chosen according to specific lifestyle requirements, but on the basis of temporary fashions.

Observation point: the veterinary surgeon

Vets treat animals when they are sick and examine animals in order to establish their good health and authorize their killing soon after in slaughter houses.

They don't do all this because they are mad, confused or wicked, but because this is required by human society, and this is what it has put into practice for milleniums, that is since it has dealt with animals.

Only in the last centuries a profession has evolved that deals specifically with animals, with their health, with their welfare; this profession, Veterinary Medicine, has been assigned with the task of carrying out practices that are considered legitimate and hopeful.

With a growing consideration for animals, it appears to become more difficult to manage such state of things.

A way of making rationally acceptable this kind of practices, could be that of distinguishing animals in "good to think" and "good to eat". That is animals to which have been attached relationship functions, according to man's use, with the purpose of stimulating the psychological aspects of human kind, and other animals that are instead used for production purposes that must satisfy especially the material aspects of man's needs.

If we should accept this vision of things, we would end up with completely excluding any kind of intrinsic value of the animal, since we would be giving it a value according only to the use we attach to them.

Therefore we cannot accept this solution and we must continue to live with the problem trying to deepen the philosophical considerations on welfarism, rightism and real human willingness to change the state of things.

Veterinary medicine can provide diagnostic and therapeutic means of great vanguard also in human medicine. These means may be applied for recovery from diseases which can be very serious and for which in the past there was no solution. At present it is possible to operate on an animal that is dying in order to revive it and permit a prolongation of its existence.

Sometimes the condition occurs, for which, even in veterinary medicine, the problem may arise whether to carry out some kind of therapeutic persistence or not.

Veterinary medicine takes care of superintending the sanitary conditions of food products of animal origin. Before being slaughtered, the animals undergo vet's examination to ensure that their general health is good, since otherwise, they could not be slaughtered. In this case, on the contrary of what happens in a veterinary clinic, it is the good health condition that leads to the suppression and not a suffering condition as in euthanasia.

This is not the place for a philosophical consideration; this is the place for the presentation of some observations that show the self-contradiction between the purposes and the practical realizations.

We have seen how it is not possible to not take into consideration, even when talking about pets, the destiny of production animals if we don't want to incur in an awfully anthropocentric choice such as that to attach importance to animals according to man's use of them.

It must be said that in regard to the animals in zootechnical productions, despite the interest for their welfare, the conditions of their lives in the last decades don't show any improvement especially due to a basic reason that nullifies all our attentions.

The economical value of the single animal has drastically dropped so that it is no more possible to address individual attentions to single animals; due to this total uncertainty of economical survival in zootechny, many traditional cattle farms, especially those nearer to nature, have closed down or will do so in the near future.

The productions merge more and more towards industrialization and loss of animal individuality, in the attempt to lower production expenses for the products that the consumer intends to buy at ever decreasing prices and perhaps with ever growing wastes.

Even though in growth, ethical brands, that care for the welfare and respect of animals, cannot prevent the widespread phenomenon that involves the clear reduction in number of traditional farms where the direct contact with the animals allowed better attentions.

In regard to pets which are the main subject of this paper, the contradictions are at first sight less evident to a public that has not got a professional kind of approach (that is all the consequent store of knowledge and sense of duty which are characteristics of professional work).

There are some chapters that need further attention.

It is becoming more and more popular with families to keep wild animals as companions in their homes.

Independently from the fact that they may have been captured in the wild or that they may have been bred in captivity, these animals have not been through the domestication process and we must imagine that their condition of life in man's environment, living in close contact with man can develop in them a constant condition of distress.

Owing to the present attentions to interests, welfare, dignity and even animal rights, such a condition should not occur, on the contrary it should be excluded right away with a dutiful precautionary approach.

Even more significant is the fact that this condition of captivity is justified by love for those animals, which leads to a further contradiction: that of causing damage to a loved one because one says to love such animals; all in accordance with the most disrespectful paternalistic rules.

Another significant observation is the increase of pure bred dogs and cats purposely purchased for simple liking or because they are considered the hip of fashion, and not in relation to a caring decision in prevision of a suitable kind of lifestyle for that particular animal.

The result is that we witness the purchase of animals, with perpetuated deformities, such as the bulldog (but there are lots of other examples), as a specific choice that satisfies a particular market.

This condition of genetic ill-treatment leads, not to an attempt to reduce those foreseeable genetic sufferings, but on the contrary, to the increase of these conditions occurring, showing a total irrational behaviour.

If one loves an animal, one should not wish the birth of animals with hereditary taints.

We need to ask ourselves what is happening, and I believe that the answer has to be found in the superficial attitude towards animals, and in the lack of information and discussion.

Emerging problems are related to the offer increase for clonation of pets carried out through the Internet.

The attempt to perpetuate the life of a pet, apart from being senseless, for the fact that it is possible to obtain an animal with the same morphologic characteristics, but it is absolutely impossible that, that particular animal will have the same kind of behaviour as the previous one, shows a level of anthropocentredness which nullifies any kind of value of the animal, since the value of individuality would be completely nullified by something that can be reproduced.

Pet therapy itself, which is based on man-animal relationship, enhancing the value of the animal, in some cases it is seen as something magic, especially when adopted with tamed wild animals which are far more stressed than domestic pets in such conditions.

Bioethics as possible solution

It is absolutely necessary to fight simplifications, to organize a discussion, to provide information, to make common and shared decisions, if we really feel we want to care about the condition of animals. For those who work with animals with a professional sense of duty, this wish becomes an absolute need.

Bioethics is the discipline that can provide a method suitable for tackling adequately and systematically the problems expressed. This is applied ethics, not theoretical, and its purpose is

to give an answer to the problems that arise in a certain place and at a certain time without claiming to have given final solutions suitable for all situations.

It is based on exchanges of points of view, of experiences and of knowledge, it accepts all legitimate values, it seeks the most common equilibrium but it makes a point of giving instructions that can be carried out in practice. It requires the formation of Bioethical Committees that can deal with general matters and specific cases.

In these Bioethical Committees, work is carried out by exponents with different interests and different competences: vets, philosophers, breeders, animalists, ethologists, lawyers, economists, educationalists and others, if need be, as permanent or temporary members or only as experts to be consulted.

This work must be carried out by the group in order to have a real exchange of information and opinions.

In a calm discussion that aims to achieve some results, critical considerations may arise; mediations can follow with temporary value that eventually allow the achievement of real objectives.

On these bases the Veterinary Bioethical Committee was founded, and since 1997 it has produced works on:

– animal transport,
– informed consent in Veterinary Medicine,
– euthanasia,
– slaughtering and ritual slaughtering,
– dangerous dogs.

For these same reasons the Bioethical National Committee for the Italian Government has dealt with animal problems discussing specific topics such as:

– animal experimentation,
– bioethics and veterinary sciences,
– pet therapy,
– ritual slaughtering,
– plastic surgery in veterinary medicine.

Informed consent in veterinary medicine

The self-contradiction in general behaviours, in relation to animals, must request the consideration of the professional vet who must try to establish what kind of client he is facing when dealing with the presented clinical case.

The morals of animal owners can be very different and go from one extreme, where the owner considers animals as things, to the opposite extreme where people think that animals have natural rights.

However, veterinary medicine has made so much progress, in regard to its technical possibilities and its availability of means, that when the moment occurs to define a diagnosis or to carry out a therapy, there is such a wide range of alternatives that it is not possible to determine a strategy without sharing the decisions with the owners.

At the same time different diagnostic and therapeutic strategies can mean a big difference in costs. The condition becomes more tragic if the possibility of euthanasia is brought up.

The Veterinary Bioethical Committee produced, some time ago, two volumes on these subject matters in the publishing series "Considerations of the Veterinary Bioethical Committee": "Procedures for an informed and responsible clinical decision. Considerations on

the so called informed consent in Veterinary Medicine" (1) and "Killing of animals. Euthanasia, means for a moral analysis" (2).

Without going specifically into the contents of the volumes, part of the specified conclusions have been incorporated in the new Code of Conduct of Italian Vets in a particular article on the informed consent:

> "Art. 29 – Duty to inform and informed consent in Veterinary practice- The Vet, on undertaking contractual responsibilities, is bound to give all information in a clear manner to the client in regard to the clinical situation and the therapeutic solutions. The Vet must inform exactly on the risks, the costs and the benefits of the different and alternative diagnostic and therapeutic routes, as well as informing on the foreseeable consequences of the eventual decisions. When informing the client, the Vet will have to consider his/her degree of understanding in order to allow him/her to give full approval to the diagnostic-therapeutic proposals.
>
> Any further request for information by the client must be fulfilled.
>
> The Vet must also fulfil any subject's information request on prevention matters.
>
> The Vet is bound to inform the client on foreseeable suffering and pain conditions of his/her animal and on the presumable duration of the professional operation.
>
> It is the Vet's duty to communicate to the client the need to carry out particular actions in order to avoid suffering, pain or prolonged illness conditions in the patient animal.
>
> The purpose of this approach consists in the attempt to give the client and its animal (in this case it doesn't make any difference whether the animal is a pet or a production animal) the chance of choosing between the different options given by the Veterinary profession.
>
> The decision will be taken according to *each single case* but always by mutual consent between client and Vet.

The work of general analysis provided by Veterinary Bioethics allows to go into the animal question, to tackle contradictions and to prevent superficial attitudes independently from the eventual conclusions.

Bioethical everyday and specific attention, through the informed consent, allows to share with an animal owner the decision that has to be made in regard to the animal, that is undertaking responsibilities both from a moral and a material point of view.

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BENEFICIAL EFFECTS OF PET RELATIONSHIPS: RESULTS OF A PILOT STUDY IN ITALY

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Some general concepts on human-animal relationships

At the end of the last Ice Age, the transition from hunting-gathering to farming favoured the process of animal domestication. The first species to make the transition from a wild to a domestic state was the wolf (*Canis lupus*) and its domestication was based on a mutually beneficial relationship with man. Until recently, archaeological findings were the only evidence to pinpoint the beginning of man's symbiotic relationship with dogs, the commonly accepted date of dog's domestication being placed at 14,000 to 10,000 years ago. However, some anthropologists suggest that the human-dog relationship could be almost as old as modern man himself (1).

In return for companionship and food, the early ancestor of the dog assisted man in tracking, hunting, guarding and a variety of other purposes. Eventually man began to selectively breed these animals for specific traits. Physical characteristics changed and individual breeds began to take shape. As man wandered across Asia and Europe, he took his dogs with him, using them for additional tasks and further breeding them for selected qualities that would better enable them to perform specific duties.

One of the most important aspects of the domestication of canids has to do with the selection of social-communicative skills (2). As an example, dogs are more skilful than great apes at a number of tasks in which they must read human communicative signals. Furthermore, wolves raised by humans do not show the same skills as domestic dogs, including puppies that have had little human contact. These findings suggest that during the process of domestication, dogs have been selected for a set of social-cognitive abilities that enable them to communicate with humans in unique ways (3). Thus, dogs able to use social cues to predict the behaviour of humans more flexibly than could their last common wolf ancestor have been at a selective advantage.

Despite the efforts of generations of ethologists and psychobiologists, until recently animals have not being recognized to possess a "mind". Historically, cognitive ethologists gained scientific acceptance between the end of the 70's and beginning of 80's (4, 5). In these years the interest for animal cognition, intelligence, consciousness passions and emotions has flourished (interested readers can refer to "Minding animal" (6) written by canine expert and bioethicist Mark Bekoff) as well as it has increased the interest for the ways in which humans interact and communicate with species with which they have the closest contact and vice versa.

The ability to communicate in the absence of a common articulate language and to modify their emotions in a reciprocal way is an essential and founding element for the ability of dogs to act as therapists. The first scientific record of such ability dates back to the 70's, when, in the laboratory of Harry Harlow at the University of Wisconsin and in the California Primate Center directed by Bill Mason at Davis, highly original research was conducted on primates in the field of ethology and psychobiology, in order to identify the selective features characterizing the relationship which is established early on between a newborn and its mother. This research was

highly revolutionary for that time. It highlighted the basic role played by the mother-infant relationship (in which both members have a reciprocal "creative" and "active" role) in shaping the emotional behaviour of the offspring. Moreover, these studies shed light on the possible mechanisms underlying the vulnerability and onset of psychiatric disorders, such as autism, and, especially, the possible outcome on neuropsychological development resulting from malfunctional bonding created during the neonatal and infantile phases. In these studies, young conspecifics (monkey therapists, characterised by an adolescent phase with a strong filial bond) were used for the recovery of juvenile monkeys with autistic characteristics. It was of interest that other species, dogs in particular, would found to be effective, while inanimate surrogates (cloth-covered plastic horse) would not (7-10).

Recent studies on humans have shown that a relationship with an animal, not exclusively a dog, can ameliorate the self-confidence and increase the learning capabilities and the motivation to interact socially.

What is pet therapy

The term "pet therapy" was coined in 1964 after a child psychiatrist Boris M. Levinson, observed positive effects while using his dog, Jingles, in sessions with severely withdrawn children. He noticed that the dog served as an ice-breaker and provided a focus for communication. Thanks to the animal, Levinson was able to establish a relationship with the child and start an effective therapy. Since then, scientists and health professionals have put Levinson's theories into practice and now a wide range of health professionals recognise what many pet owners have known for years – i.e. that pets can be good for our health and well-being.

It is important to notice that nowadays professionals discourage the term "pet therapy" because it actually refers to animal behaviour training programs and prefer to distinguish between:

– *Animal Assisted Activities* (AAA) provide opportunities for motivational, educational, and/or recreational benefits in order to enhance quality of life of some human categories such as blind and physical or psychic handicapped persons. AAA are delivered in a variety of environments by specially trained professionals, para-professionals, and/or volunteers in association with animals that meet specific criteria.

– *Animal Assisted Therapies* (AAT) are goal-directed interventions in which an animal meeting specific criteria is an integral part of the treatment process. AAT are delivered and/or directed by a health/human service provider working within the scope of his or her profession. AAT are designed to promote improvement in human physical, social, emotional, and/or cognitive functioning. They are provided in a variety of settings and may involve groups or be individual in nature. This process is documented and evaluated.

In Italy pet therapy has been recognized as official care by a Legislative Decree (DL.vo issued on February 28th 2003; following an agreement between the State and the Italian Regions). For the first time in our country, this Decree sanctioned the role that an animal could have in the emotional life of a person and the therapeutic benefits derived from pet animals.

Who can benefit from pet therapy

People who usually can benefit from pet therapy are:

– *Children*
Pet therapy decreases children's stress and anxiety about illness, injury and hospital experience. Interacting with a pet can sometimes enhance recovery following a serious illness. It can change behaviour, create a sense of responsibility and even improve a child's ability to participate in therapeutic treatment leading to attainment identified goals and objectives. Children are often extremely trusting and easily achieve a level of intimacy with animals. This special bond contributes to pets' effectiveness as co-therapists (11).

– *Elderly persons*
In the institutionalized elderly there is evidence that pet therapy may reduce depression, blood pressure, irritability and agitation, and may increase social interaction. In an epidemiological study performed on people that had suffered from infarction, the presence of a pet was found to have a positive effect on survival (12). In Alzheimer's disease there is evidence that the presence of a companion animal may increase social behaviours such as smiles, laughs, looks, leans, touches, verbalizations, name-calling, or others. Moreover pet therapy has been shown to reduce loneliness and depression in residents of long-term care facilities, particularly in people with a prior history of pet ownership. The presence of a pet has also been found to lead to increased verbal interactions among residents (12, 13).

– *Psychiatric patients*
There is evidence that presence of a pet among psychiatric patients promotes social interactions (14). In people with schizophrenia pet therapy may lead to improved interest in rewarding activities as well as better use of leisure time and improved motivation. There is also evidence of improvement in socialization skills, independent living, and general well-being. In a large, well-designed study, hospitalized patients with a variety of psychiatric disorders were found to have reduced anxiety after a single session of Pet therapy (14). For most, the benefits were superior to those of a session of regular recreation therapy.

Educational activities promoted by pet relationships

According to some reports, pets, with their morphological and behavioural diversity, could solicit the child in the formation and enrichment of its imaginary world, offering him/her more than one model for his/her elaborative processes and strengthening his/her imagination. Moreover, the interaction with the animal diversity, or the simple referring to it, could help the child in coping with a multifaceted world, transforming the diffidence in curiosity and tolerance and decreasing widespread fear. The act of taking care of a companion animal usually decreases generally aggressive behaviours, negligence, little helpfulness. Pet relationships increase affectivity, fortify the epimeletic tendency of a child, the capabilities to take care, to help and protect someone, and decrease general disorganization, low attention to external and inner world. Moreover, this relationship helps a young boy/girl in having a positive behaviour in all the daily activities (11).

Usually, pets have juvenile characteristics able to stimulate communication and to solicit children to play activities. Pet owners taking care of their animals, give rise to an epimeletic behaviour and children observing this situation carry out an identification process by which they come to play the role of an adult. The "encounter" with the animal can be of great help in shaping the emotional ability of the child. This can be achieved because the relationship with

the animal has an emotional and empathic connotation and eventually leads the child to learn how to self-regulate its arousal states, in order to effectively interact with the pet.

An increasing motivation and attention has been observed, for example, when pets are regularly in school classes in which children with mental retardation are present. Pet became the centre of attraction ameliorating, at least in part, the learning capability deficits of these children. It is well known the study on an autistic girl that learned to count up to three just to start a game in which her dog was involved in (11).

Animals used as pet therapists

The animals most commonly used for pet therapy are:

– *Dog*
  This is, by large, the most frequently used animal as co-therapist, both with children, adults and elderly people. By soliciting play, dogs arouse patients and demand interactions, in addition to offering company (2).

– *Cat*
  It is enrolled as co-therapist for its independence and the easy way to take care of it. It is preferred by people living alone or having an age or some pathologies that limit their movements (15).

– *Hamster and rabbit*
  To observe, to pet and to take care of these animals could bring great benefits, especially to children having a hard time in their life.

– *Horse*
  Horses are mostly employed for medical, rehabilitative and psychological-educative hippotherapy practised in equipped facilities by the help of a well trained staff. Autistic children, Down syndrome children, disabled persons with behavioural and motor dysfunctions can benefit from hippotherapy.

– *Bird*
  Studies performed on groups of elderly people have evidenced the beneficial effects of the usually taking care of birds, in particular parrots.

– *Fish*
  It has been noticed that the observation of a fish in an aquarium might help in reducing tachycardia and muscles strain, acting as an anti-stress.

– *Dolphin*
  These animals have been employment as co-therapists in the case of depression and mental and emotional disorders. The dolphin therapy can improve autistic patients' psychological status and social adaptation.

– *Donkey, goat and cow*
  These are domestic animals that can also be employed in pet therapy practises.

By and large, domestic animals, particularly small mammals, should be preferred as Pet Therapists as they are those that have been selected for their ability to interact socially (and emotionally) with humans.

Professional categories involved in pet therapy: the working group

In Pet therapy, the activity performed by the "animal therapist" towards the "human patient" is very complex and to be successful, above all, it requires the contribution of many professional figures.

For this reason, every Pet therapy experience is the results of the combined effort of a cross-disciplinary team made up of various professional categories. These categories interact and bring their own specific contribution in a complementary way.

As operators, the members of the team work personally at the design, at the evaluation of programs and at the execution of activities and therapies. In particular, it is important that these activities do not result stressful for the animal itself (16).

Ideally, the Pet therapy team should be made of all (or most) of the following figures:
– Physician;
– Psychiatrist;
– Psychologist;
– Rehabilitation therapist;
– Social worker;
– Nurse;
– Teacher;
– Pedagogue;
– Vet;
– Ethologist;
– Professional dog trainer;
– Pet conductor.

Pilot study on AAA and AAT activities in a sample region of Italy: Emilia Romagna

The increasing interest in pet therapy and the lack of guidelines that formally regulate the therapies performed with animals, has raised the need to document the activities that are being undertaken in Italy under this label. This initiative involves the Istituto Superiore di Sanità and the Faculty of Veterinary Medicine of the University of Bologna, and is aimed to chart all the initiatives in this field in continuous expansion, in the Italian region Emilia Romagna.

The goal of our study was to identify both the common and the discriminating factors between several operators recognised in the Emilia Romagna territory and to collect them in macro-groups of "certification". To this purpose we selectively identified a number of parameters, such as the professional profile of pet operators, the formative background of animals employed, the typology of users, the type and the degree of the handicap, the type of structure in which the activities are performed and the institutions involved.

A first consideration on the professional profile of the operators brings out the fact that in the region Emilia Romagna the majority of people belonging to this category has the *Referee Pet Operator* certificate or the *Pet Partner Operator* certificate, qualifications obtained after attending the *Referee in Welfare Zooanthropology* course organized by the SIUA (Scuola di interazione uomo-animale: Man Animal Interaction School). A smaller group of operators in Emilia Romagna has an AIUCA (Associazione Italiana Uso Cani d'Assistenza: Italian

Association Use of Dog for Assistance) certificate and performs activities with equine horse therapists. Finally, an additional group enlist an operator having a Delta Society certificate who interacts with veterinarians (Figure 1).
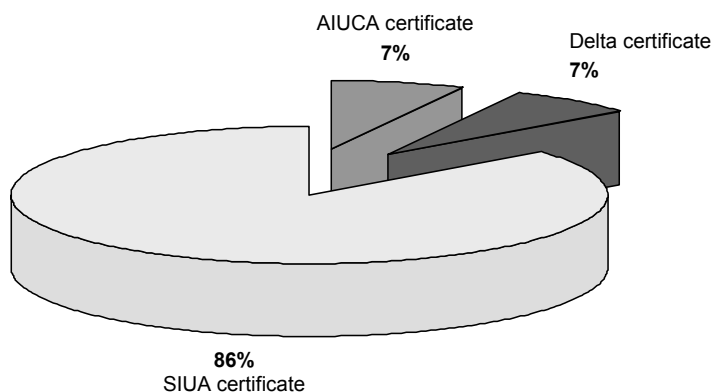
**Figure 1. Professional certification of pet therapy operators**

Pet operators, for the most part, are graduates in Pedagogy, Veterinary Medicine, Psychology, Environmental Sciences, Natural Sciences, Medicine, Biological Sciences, Pharmacy, Geology, or high school diploma, Dog trainer, operators in social services, hold Diplomas for Athletic/ Physical trainer (Figure 2).
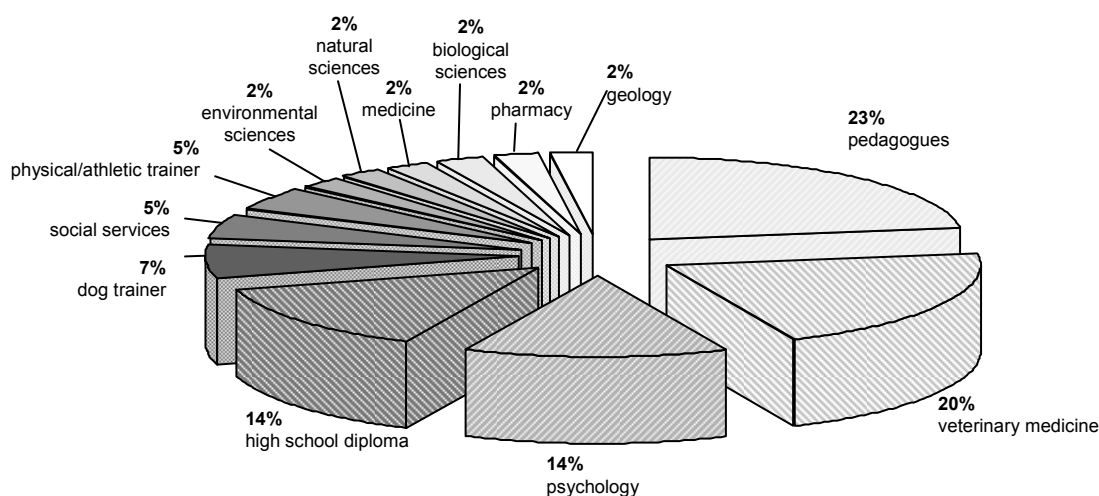
Figure 2. Education/main occupation of pet therapy operators

Many projects of AAA and of AAT are listed as zooanthropology projects for children (Zooantropologia Didattica, ZD). These are projects of Pet Education devoted to children aged 2-16, some with handicap of different types. These operators, in addition to the certificates in

Referee in Welfare Zooanthropology or *Certified Pet Partner Couple*, also obtained a certificate in *Referee in Didactic Zooanthropology* at the SIUA or by SCIVAC (Società Culturale Italiana Veterinari per Animali da Compagnia: Italian Cultural Society of Companion Animal Vets) (Figure 3).

16%
ZD

2%
AAA + residential dog

23%
AAA

59%
AAT
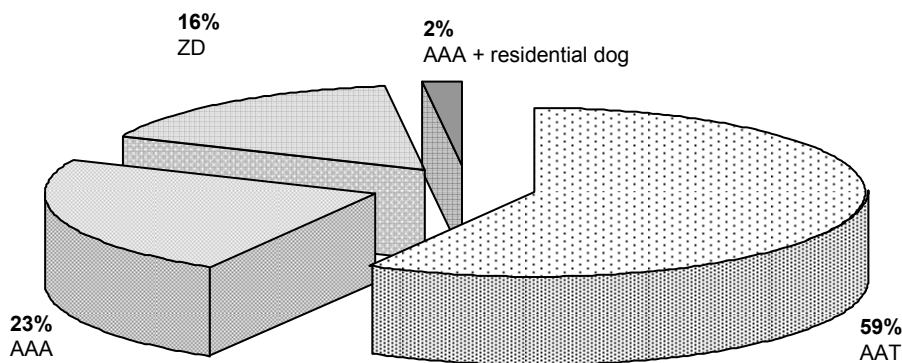
**Figure 3. Relative occurrence of AAT/AAA projects
(in Emilia Romagna 26 ZD projects have been reported)**

In some cases the operators with a certificate in *Certified Pet Partner Couple* and *Referee in Welfare Zooanthropology* are also *Dog Educators*, certificate obtained at the SIUA.

As for training courses involving dogs, subjects coming from amateur farms, but also dogs coming from kennels and professional farms have attended the *Certified Pet Partner Couple* Course. Following this training, dogs obtain the SIUA certification for A, B, C, D category.

On the other hand, to become *Referee Pet Operator*, the dog has to attend a course of *Basic Education* and of *Education to Relationship*. This allows it to take part in a working group (without forming a certificated couple).

One case of *Certification of Dog for Assistance and Therapy* has been documented at the *Assistance Dog Institute*, Rounert Park, California and one dog was found certified by *Delta Society*.

Sporadically, animals belonging to various species such as dwarf rabbits, California rabbits, dwarf Tibetan goats (coming from farms unharmed from brucellosis), cats, turtles and tortoise have been reported as being used as pet therapists (Figure 4).

The typology of AAA and AAT users is represented, mostly, by children and elderly, followed by adolescents and adults. As for children, there are subjects with verified diagnosis of hiperactivity, deficits in learning, Down syndrome, West syndrome, Rett syndrome, mental delay, speech and communication disorders of different levels (degree), as well hospitalized children.

As for adolescent, these are teens with physical and/or psychic handicap, experiencing social unease and maladjustment.

Adults are psychiatric patients with schizophrenic symptoms and relational disorders, in addition to cases of autism, psychosis, mental handicap, premature senile dementia and mental retardation. Carriers of psychiatric pathologies associated with cognitive deficits as well patients in semi vegetative status due to severe brain lesions, patients with post-traumatic psycho-physic disabilities, ex drug abusers with confused and depressive states.

In the case of elderly people, the pathologies more often found are senile dementia, Alzheimer's disease, confused and anxious states, mental and physic disabilities, motor disorders.
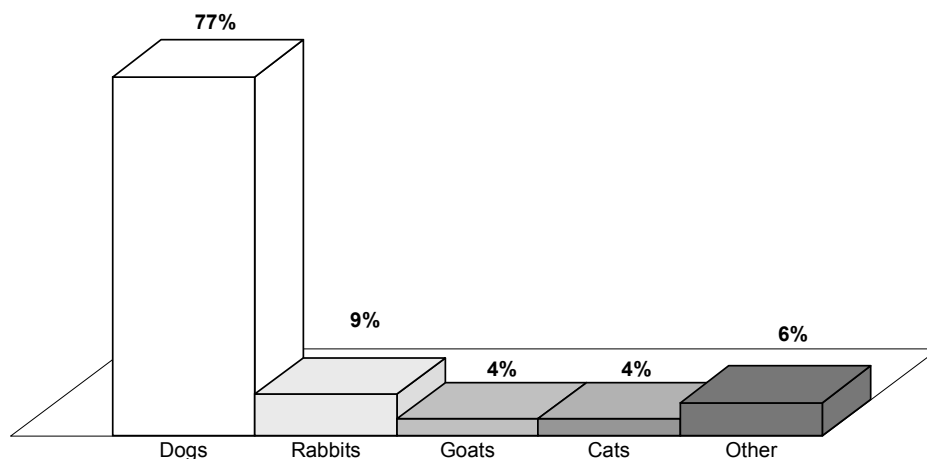
Figure 4. Animals usually used as pet therapists

The structures hosting pet therapy projects are public structures such as nursery schools, kindergarten schools, elementary schools, middle schools, high schools, residential homes for adolescents without a family, residential houses, daytime centres for disabled, institutes of public assistance and charities (socio-rehabilitative daytime centres and sheltered houses for elderly), the Judicial Psychiatric Hospital of Reggio Emilia. Between the private structures we can enlist: nursery schools, private structures for disabled people, private houses with children and adolescents. Hospital structures in which pet therapy is performed are: the Judicial Psychiatric Hospital of Reggio Emilia, and the Paediatric ward "Gozzadini" of the S. Orsola Hospital of Bologna. In some other Hospitals, such as the Rizzoli Hospital of Bologna and "The house of Awakenings Luca De Nigris" (Department of Neurosciences, Maggiore Hospital and Bellaria Hospital), AAT projects have been scheduled to start but have not yet been activated (Figure 5, 6).

The institutions involved included numerous towns with surroundings and villages – Bologna (Calderara di Reno, Casalecchio di Reno, Castel San Pietro, Granarolo dell'Emilia, Osteria Grande, San Giorgio di Piano, San Giovanni in Persiceto, Zola Predosa), Modena (Castelfranco Emilia, Carpi, Castelnuovo Rangone, Campogalliano, Formigine, Sassuolo), Reggio Emilia (Bagnolo in Piano), Ravenna –, the Province of Bologna, the Region Emilia Romagna, the Office for Animals Rights of Bologna, The Society of Transport of Ravenna, the Faculty of Pedagogy of the University of Bologna, the Institute Charitas of Modena, some social cooperatives, the Local Health Unit of Modena, the Services of Paediatric Neuropsychiatry of the Local Health Unit of Modena.

In the region Emilia Romagna, from 2001 to 2006 included, 37 AAA projects were registered as well as 92 AAT projects, 26 zooanthropology projects for children and adults, 3 AAA projects (one is still ongoing) forecasting the permanent custody of the dog to the structure in which the activities are performed.

Forty-one operators have been involved: 37 work for 7 groups registered and 4 work as individuals. The total number of animals involved is greater than the one of the animals really used in the projects carried out. However, 56 animals overall were used, 43 of which were dogs. In relation to the institutions involved in the project from 2001 to 2006, 26 are institution for the elderly, 15 institutions for adolescent disabled, 14 elementary schools, 11 nursery schools, 5 kindergarten, 2 middle schools, 2 high schools, 1 private nursery school, 1 nursery school breast-fed section, a veterinary surgery of Local Health Unit at the municipal kennel, 1 judicial psychiatric hospital, 1 general hospital, 14 private houses (Figure 5).
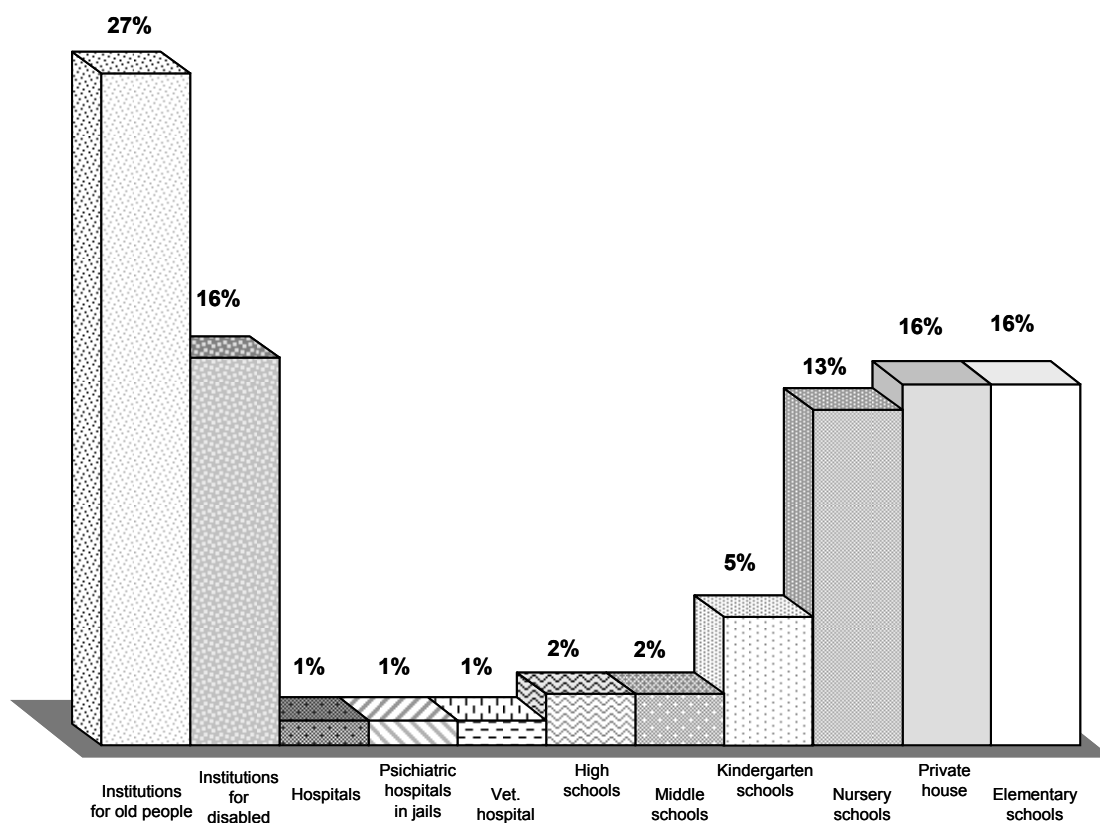
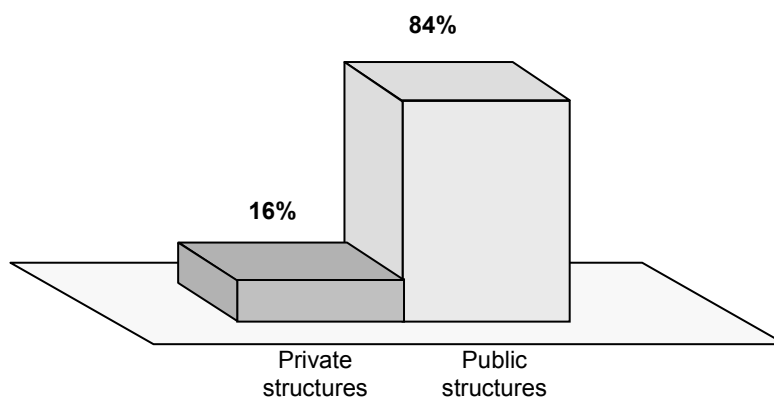**Figure 5. Main structures hosting AAA and AAT activities**

Figure 6. Main structures hosting AAA and AAT activities

The smallest number estimated of users profiting by services delivered between 2001 and 2006 (excluding projects devoted to primary and nursery schools for which it could be too arbitrary to establish a minimum number of users) reaches the number of 407.

Conclusions

Overall, Pet therapy and AAA have revealed, with time, their potential ability to heal as well as to provide opportunities to enhance the quality of the life of people with physical and mental disabilities (17).

At the institutional level, growing efforts have raised attention in pet therapy and AAA. However, no established methodologies are presently available for the therapeutic exploitation of animals, but the pressing need to help affected categories, especially children, has stimulated scattered efforts at an explosive pace.

The Istituto Superiore di Sanità, which plays an important advisory role in the Italian health system, has being taking an ever increasing role in attempting to regulate these activities by e.g. selecting good-quality training activities at the public academic system level (mainly University courses and Masters) and by sponsoring a few pilot experiences.

In this rapidly growing field, we are attempting to fill the need for i) identifying standard curriculum for trainers, avoiding spontaneous initiatives; ii) establishing, by means of a scientific consensus (both at the European and national level) a draft of guidelines to be implemented in the near future in selected centers, endowed by good scientific and clinical credentials; iii) promoting, at the international level, university research in pet/humans relationships, in order to study how dog's emotions are communicated to humans and other dogs.

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Useful web sites about pet therapy

http://www.iss.it/neco/Pett/cont.php?id=15&lang=1&tipo=5/
http://www.ministerosalute.it/dettaglio/phPrimoPiano.jsp?id=118
http://www.vet.unibo.it/Medicina+Veterinaria/Facolta/Strutture+di+servizio/terapie_attiv_assist_animali.htm

HUMANS AND THEIR ENVIRONMENT: ETHICAL ASPECTS

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Historical background

Until recently, Western culture has been predominantly indifferent towards the ethics of nature and of animals: for most of the Western history the "moral status" of animals did not seem to be noteworthy and philosophers did not write extensively about it. Few authors took the issue into account. Their typical approach was similar to Kant's perspective. The latter approach recognizes a moral value only in humans: nature and animals deserve respect, but are morally indifferent (1).

On the contrary, in some Eastern systems of thought, historically animals are accorded a moral status and a great respect. The Jains of India, for example, hold that all life is sacred, drawing no sharp distinction between human and nonhuman life. They are therefore vegetarians, as are Buddhists (2).

In Western culture a new debate on environmental and animal ethics developed at the end of the eighteenth century and during the nineteenth century owing to several circumstances (3).

The utilitarian philosophy, led by Jeremy Bentham and John Stuart Mill, challenged the traditional exclusion of animals from moral concern. According to utilitarianism, morality is fundamentally a matter of seeking to promote happiness and prevent suffering. Bentham suggested that there is no reason to limit moral concern to human suffering and that disregard for animals is a form of discrimination analogous to racism (4).

Another important contribution was the publication of "On the origin of the species" (1859) by Charles Darwin, suggesting that men and apes are similar because they inherited many characteristics from the same ancestors. The differences that exist, according to Darwin's theory, are matters of degree, non of kind (5).

During the twentieth century, and particularly in the last decades, environmental and animal ethics have been widely studied and discussed by scientists, philosophers, moralists and theologians. Ecological knowledge of environmental processes and systems, and the perception of risks, caused by an ever increasing influence of humans upon their environment, have undoubtedly contributed to the growth of this interest. As regards animal ethics, the debate has been fed particularly by the publication of Peter Singer's "Animal liberation" in 1975, which stimulated a wide-ranging debate (6).

Different theoretical principles about the moral value of the relationships between man, nature and environment have been proposed. Depending on the principles taken as references, usually the various ethical perspectives are gathered in two main currents: anthropocentrism and non-anthropocentrism. Nevertheless, deep diversities distinguish not only the two perspectives, but also different trends within both perspectives (7).

Anthropocentric ethics

Anthropocentrism is a broad concept in ecophilosophical thinking. The dictionaries' definitions of the term "anthropocentric" refers to any view that regards humans as the most important and central being in the universe (8, 9).

The assumption of human importance and centrality in the larger scheme of things has been widely accepted both by religious and secular views. Historically, the anthropocentric perspective is rooted in Aristotle's philosophy.

Aristotle taught that 'nature does everything for a purpose', and so, just as plants exist to provide food for animals, animals exist to provide food and aid for humans. In the following centuries, traditional Western moral philosophy has been concerned mainly with human action in relation to other human beings, and has frequently considered nonhuman beings and nature as a whole to be mere means to human ends, and not ends in themselves (10).

The difference between animals and men and the privileged moral status of humans are explained mainly by two characteristics: for Aristotle, and for many other philosophers, the difference is that humans alone are rational; Christian faith adds that only man is made in the image of God.

A multitude of versions over and above the most general formulation of anthropocentrism have been proposed.

Weak anthropocentrism

"Weak" anthropocentrism is less strict than other interpretations of anthropocentrism. It proposes a sort of "noblesse oblige" attitude of humans with respect to nature. According to "weak" anthropocentrism, this attitude would enhance and ennoble human life, and at the same time would protect the environment. Bryan G. Norton is one of the best known philosophers who supported this position.

Norton and other anthropocentrists insist on man's duty to preserve nature for the use and the enjoyment of future generations, introducing in the debate the important notion of "responsibility" (11).

Other authors (e.g. Eugene C. Hargrove (12) and Mark Sagoff (13)) prefer to think that the intrinsic value in nature is mainly aesthetical (and therefore human-centred) than ethical in character.

Catholicism

According to Catholicism the dignity of the human person is rooted in his creation in the image and likeness of God. Man received a mandate to govern the world with justice and holiness, relating himself and the totality of things to the Creator. In this sense, catholic environmental ethics is properly understood not as being anthropocentric, but "theocentric". According to Catholicism, men have to labour, throughout the course of the centuries, to better the circumstances of their lives. The Church appreciates the advantages that result from science, technology, agriculture, industry (14). At the same time the Church requires their proper application, since this potential is not neutral: it can be used either for man's progress or for his degradation (15). Respect for humans must also be accompanied by a necessary attitude of respect for other living creatures. Even when thought is given to making some change in them, one must take into account the nature of each being and of its mutual connection in an ordered system.

The social doctrine of the Church underscores that the goods of the earth were created by God to be used wisely by all: they must be shared equitably, in accordance with justice and charity (16).

According to Roman Church a correct understanding of the environment prevents the utilitarian reduction of nature to a mere object to be manipulated and exploited. At the same time, it must not consider nature as something absolute and place it above the dignity of the human person himself.

The Catholic Magisterium underlines human responsibility for the preservation of a sound and healthy environment for all. Care for the environment represents a challenge for all of humanity. It is a matter of a common and universal duty, that of respecting a common asset.

Responsibility for the environment, which is the common heritage of mankind, extends not only to present needs but also to those of the future (17).

Kantianism

Although Kant lived before the classical utilitarians (see above), his ethics is best understood as a critique of the utilitarian approach (which after all was not entirely new with Jeremy Bentham and John Stuart Mill): in contrast to the utilitarian emphasis on sentience as the basis for the moral status, Kant regarded the capacity for experiencing pain and pleasure as morally insignificant. Instead, he emphasized that the connection between morality and reason is what separate human beings from the rest of the animals and what makes us subject to the moral law. Our capacity for rational thought both entitles us to treatment and rights to which animals are not entitled, and imposes on us obligations that animals do not have.

Kant's moral philosophy regarding the treatment of animals draws inspiration both from Aristotle and from Thomas Aquinas. The three authors emphasize rationality as the defining characteristics of being human and holder of a moral status. Kant objected to cruelty against humans because of the deleterious effects on human themselves: who is cruel to animals becomes cruel also with men.

Kant's animal ethics draws inspiration from classical philosophy, but his speculations are in many ways original. According to Kant humanity exists as an "end in itself": this is a postulate that does not need to be proven by other principles.

Like Aristotle and Aquinas, Kant views animal and the whole nature as existing to serve human interests: in particular animals exist merely as a means to an end, and that end is man (18).

Contractarianism

In the last half of the twentieth century another view, usually called 'contractarianism' emerged as rival of utilitarianism. According to contractarianism, morality rests on agreement of mutual benefit: morality arises within a community when each person agrees to 'play the social game', respecting other people's rights and interests, provided others will do so as well. This agreement makes social living possible, and everyone benefits form it. But animals are unable to participate in such agreement, so they do not fall into the sphere of moral protection (19).

Nonanthropocentric ethics

At the beginning of the twentieth century, some authors suggested the need for completely new, non-anthropocentric ethics. Many of them (e. g. Richard Routley (20)) considered traditional ethics and philosophies inadequate to respond to modern problems, and particularly to environmental problems. They suggested the necessity to overcome traditional ethics and philosophies, or at least an enlargement in order to give moral stature to animals and to the environment. Other authors (e. g.: John Passmore) replied that normal western ethics is entirely adequate to address contemporary environmental problems, since human actions that directly degrade the environment also indirectly harm human beings. According to Passmore, and to other philosophers who propose an anthropocentric perspective, we do not need new ethics, but a steadfast commitment to our familiar set of human values (21).

The debate developed particularly in the second half of the century, when the degradation of environment stimulated the conviction that new models were needed to govern human actions affecting the environment. During this period various models of environmental ethics that embraces biocentrism have been proposed by several authors. At the same time environmental movements, concerned about various types of environmental degradation, were established in many western nations. Many of them were prompted, in the early sixties, by the publication of Rachel Carson's book "Silent spring" (22). The book is a widely-read account of the ways in which pesticides damaged the environment and led to an increased awareness of the environmental issues on the part of the public.

Two main components characterize all environmental non-anthropocentric ethics: the holistic view and the extension of moral considerations, up to now allowed to humans only, to nonhuman entities (23).

Holism refuses traditional conceptions, which recognize for man a central role in nature, and sees the planet as a community of life-forms in which each contributes to and depends upon all the others.

"Extensionism" proposes an enlargement of traditional ethical systems in order to include also environmental and animal ethics.

Utilitarians and the sentientist tradition

Critics of rationalist tradition refused the ability to reason as the criterion for admission to the moral community. Jeremy Bentahm, John Stuart Mill and, among contemporary utilitarians, Peter Singer include animals in the moral community: they maintain that sentiency determines who does, and who does not, belong.

Jeremy Bentham, who developed the classical theory of utilitarianism, averred that the capacity of suffering is one of the most important features of moral standing. Singer widens this approach and considers that all "sentient" beings should be accorded moral consideration, and that moral consideration should vary in proportion to the ability to suffer (24).

Therefore, sentience theory aims to maximize pleasure and minimize pain in all sentient living beings. To avoid "speciesism", the sentientist theory proposes that an "egalitarian view": whatever treatment is good, for example, for Alzheimer patients whose senses wane, is appropriate for animals with similar sentience (25, 26).

Biocentrism

Kenneth E. Goodpaster, one of the best-known exponents of biocentrism, argues that pleasure is not and end in itself (as Bentham suggested), nor is pain an intrinsic evil, since the capacity to experience pleasure and pain evolved among animals as a means to preserve their lives. Hence the capacity to live, not the capacity to suffer, ought to be the criterion of "moral considerability" (27).

Like zoocentrism, biocentrism considers that only individuals possess characteristics that can serve as a criterion of inherent worth and therefore grants moral consideration.

Biocentrism draws inspiration from Arthur Schopenhauer and Albert Schweitzer, although less deliberately than animal liberationists follow Bentham. The theory has been developed particularly by Paul W. Taylor (28). Usually biocentrists endorse conation (the quality of striving, whether consciously or not, toward a goal, as the criterion of moral value). The most radical biocentrists extend this criterion to plants: according to them, plants, as well as animals, are "teleological centres of life" since they strive to grow and to reproduce.

Biocentric theories usually view the environment as fragile, limited in resources and vulnerable, even if biocentrism does not directly address contemporary environmental problems: the extinction of species, the degradation of ecosytems, soil erosion, water and air pollution, are not directly dealt with by biocentrism.

The main beliefs of biocentrism can be summarized in four points.

The first is that humans are considered members of earth's community of life in the same sense as, and on the same terms that, other living things are member of that community.

The second is that humans, along with all other species, are considered integral elements in a system of interdependence such that the survival of each living thing, as well as its chances of faring well or poorly, is determined not only by the physical conditions of its environment, but also by its relations to other living things.

The third is that all organisms are considered teleological centres of life in the sense that each is a unique individual pursuing its own good in its own way.

Finally, humans are considered not inherently superior to other living beings.

Ecocentrism

There are also attempts to stretch normal theories even further, so that they will encompass every element of the ecosystem: ecocentrists assert that even "rocks have rights".

The "land ethics" sketched by Aldo Leopold has been the inspiration for the modern American environmental movement and the point of departure of a non-anthropocentric, nonindividualistic and ecocentric moral theory. Leopold's theory draws inspiration form the moral philosophy of David Hume and Adam Smith. According to Leopold "a thing is right when it tends to preserve the integrity, stability and beauty of the biotic community, and wrong when it tends otherwise". Therefore, it would be right to kill deer and fell trees for the good of the biotic community, but it would also be right to undertake draconian measures to reduce human overpopulation (29).

Ecocentrists underline that, from an ecological point of view, individual organisms are internally related and mutually defining: nature, mother and nurturer of life, seems indifferent to individual life. Nevertheless, ecocentric environmental ethics, although providing for the possibility of moral consideration or wholes, does not completely disenfranchise individuals. Ecocentrism is holistic as well as (not instead of) individualistic: holistic concerns may eclipse individual ones. Social evolution consists in a series of additions rather than replacements. The moral sphere, growing in circumference with each stage of social development, does not expand

like a balloon, leaving no trace of its previous boundaries. It adds, rather, new rings, new "accretions", as Leopold called each emergent social-ethical community. The discovery of the biotic community simply adds a new outer orbit of a membership and attendant obligation. Our more intimate social bonds and their attendant obligations remain intact. Thus we may weigh and balance our more recently discovered duties to the biotic community and its members with our more venerable and insistent social obligations in ways that are familiar and humane (30).

Inherentism

Like the sentientist tradition, inherentism is egalitarian in spirit, but unlike the former tradition, inherentism identifies individual, not mental states as the locus of ultimate value. This kind of value individuals possess is modelled after Kant's idea of "end in itself". Individuals that have inherent value have a morally significant value in themselves, apart from the possible usefulness to others and independently of the episodic or overall value of their mental states.

Inherentism differs from the position of Kant and his followers in a fundamental respect: whereas Kantians deny that nohuman animals exist as ends in themselves (or have inherent value), inherentist maintain that other animals have this same moral status. Moreover, inherentism's advocates see themselves as offering a philosophical foundation of human and animal right, arguing that the individuals who have inherent value, whether humans or not, also possess the basic moral right to be treated with respect (or, alternatively, to be treated as ends in themselves).

Inherentism can be considered an attempt to synthesize what, according to its advocates, is the best feature of the rationalist tradition (that is: equal value of the individuals) with what, according to its advocates, is the best feature of sentientist tradition (that is: non-speciesism) (31, 32).

The "New paradigm"

The "New Paradigm" has received particular recognition within the United Nations (and some of its Agencies, e.g. UNESCO), the World Health Organization and several Nongovernmental Organizations. Wide-ranging presentations and discussions about the "New paradigm" took place also in the framework of UNESCO conferences (33).

According to the "New Paradigm", traditional religions failed in producing global ethics. The "New Paradigm" is not a religion, but a kind of non-anthropocentric new ethics and new spirituality which aims at the harmony of man in nature. In this perspective, valid elements from traditional philosophies and religions are not completely refused, instead the are brought together to form a new global ethics paradigm. For example, imperturbability is taken from Buddhism, respect for animals from Hinduism, the virtue of charity form Christianity, the virtue of justice form Islam: according to the "New Paradigm" each religion, in itself, is unable to solve problems and to answer questions that come from humankind and nature. Traditional religions are considered dogmatic, and therefore it is necessary to replace them with a culture of peace, promoting sharing, community decision making, participative democracy, negotiation, freedom, justice, equity. The "New Paradigm" broadens the stakeholder concept: it includes biosphere and future generations, even if it is impossible to communicate with them.

The "New Paradigm" aims at the definition of a common area of conduct which identifies basic ethical principles acceptable by all components of the emerging modern global society. This common background can be found in global well-being and in an environmentally sustainable development.

According to the environmental ethics proposed by "New Paradigm", it is necessary to counteract primarily overpopulation, environmental degradation, environmental pollution, uncontrolled industrialisation, production of transgenic foods, social injustice, intolerance. All these actions are necessary to reach the harmony of nature and with nature. The concept of harmony with the earth is similar to the "Gaia theory" proposed by James Lovelock (34), while the notion of sustainable development is borrowed from the well-known report "Our common future" issued by the World Commission on Environment and Development (35). The "New Paradigm" considers sustainability a crucial theme: we have reached an intolerable ecological situation, which prevents from the achievement of global well-being. A turnabout is urgent in order to prevent further degradation and to promote global well-being for all. Sustainability is interconnected with the notion of "Quality of life". This is a wide-ranging concept that can be defined as "the perception by the individual of his position in life, within the context of the culture and system of values in which he finds himself, and in relation to his goals, expectations, models and interests". Quality of life includes: physical health, psychological health, independence, social relations, personal beliefs and context (economy, security, participation, freedom, and other aspects). Pillars of this approach are: human rights, responsibility, democracy, peace, transparent negotiation, intergenerational equity. Those values are required as a basis for a readjustment between man and nature.

A comprehensive plan of action of the "New paradigm" in environmental ethics is presented by Albert Gore in his book "Earth in the balance. Ecology and the human spirit". Embracing the "New Paradigm" in environmental ethics, Gore argues that only a radical rethinking of the relationships between man and the environment can save the earth for future generations.

General remarks

Mankind is part of nature and the quality of its life depends on a proper relationship between individuals in their community and in the environment where they live. This relationship is dynamic.

One of the most striking aspects in the debate concerning the moral status of animals is that it is possible to reach radical ethical conclusions by invoking only the most common moral principles. The idea that it is wrong to cause suffering, unless there is a sufficient justification, is one of the most basic moral principles, shared virtually by everyone. Yet the application of this principle leads to a wide range of conclusions, even to refusing to farm animals, since in modern farms they suffer considerable pain.

Other arguments appeal to less common notions. For example 'speciesism'. Just as racists unjustifiably give greater weight to interests of the members of their own race, speciesists unjustifiably give greater weight to interests of their own species. This line of thought suggests that animals may be treated differently from humans only when there are concrete differences from humans. For example, it is may be permissible to admit only humans to school because human can read and other animals cannot. But in cases where there are no relevant differences, they must be treated alike, since they are morally 'equal'.

Such arguments have, of course, stirred lively oppositions. According to traditional thinking, morality is fundamentally a human institution.

Values and duties

The debate on the moral status of the various components of nature is usually based on assertions that involve the notion of "value". Sometimes the debate is complicated by the different meanings ascribed to this term: intrinsic worth, usefulness for individuals or society, symbolic worth, and also cost (economic "value") (36).

A considerable part of the debate on environmental and animal ethics has referred also to the concept of "duty". Direct and indirect duties are invoked.

Direct duties are owed to identifiable individuals, and those to whom they are owed are prima facie entitled to demand compliance. Direct duties have correlative rights. This entitlement can be expressed in terms of individual rights.

Indirect duties involve certain individuals or things, but they are not duties owed to them. For example, we have the duty to respect another's property, but we have no duties with respect to the property: the duty involves the property, but the duty is owed to the owner, not to the thing owned.

The distinction between direct and indirect duties can be used to define the moral community. On this account the moral community consists of all and only those individual (a) who have direct duties or (b) to whom direct duties are owed.

Duties are also related to responsibilities and to posterity's interests, since actions may have irreversible effects on the environment.

Rationalist and sentientist tradition

According to the rationalist tradition the capacity to reason is decisive: only those who have this capacity belong to the moral community. This view is often traced back to Aristotle.

Critics of the rationalist tradition seek an alternative to the capacity to reason as the criterion for admission to the moral community. According to Jeremy Bentham, John Stuart Mill and, among contemporary utilitarians, Peter Singer, "sentiency" (that is, the capacity to experience pleasure and pain) determines who does belong to the moral community. As Bentham writes, in a frequently quoted passage, "The question is not, can they (that is, non-human animals) reason? Nor, can they talk? But: Can they suffer?"

If sentiency serves as the test for admission, then the moral landscape is radically transformed. At least some of our duties regarding non-human animals emerge as direct duties, that is to say duties we owe to these animals themselves.

To resist this extension of direct duties to animals other than human beings, on the ground that non-human animals do not belong to Homo sapiens, thus emerges as a moral prejudice cut from the same defective cloth as racism. Like this prejudice, this newly recognized one (speciesism, as it is commonly called) attempts to justify moral differences simply on the basis of biological differences.

The egalitarianism central to what might be called "the sentientist tradition", when coupled with utilitarian theory (as it customarily is), gives rise to counterintuitive results. For example, the murder of innocent human beings seems to be permitted by the theory, if the aggregate balance of good mental states is thereby achieved, as it may well be in particular cases. Given this approach there is nothing wrong with a matador's painfully draining the life from a bull, for example, provided only that enough people find the spectacle sufficiently pleasant. To protest that "it is immoral" to take pleasure in such a barbarous custom is an objection that is unavailable to utilitarians since whether this is immoral is itself an open question, given utilitarian theory.

When considering the relation of humans with the environment and with animals, we are falling into a logical fallacy if we regard ourselves only as "managers" or only as elements in a complex ecological net. Man is a part of and apart from nature at the same time (37).

In conclusion, it should be pointed out that, even the most radical forms of animalism and environmentalism, ultimately focus on humans whenever their intention is to actively foster and protect awareness as regards living species.

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ANIMAL WELFARE, ANIMAL MINDS AND ANIMAL INDIVIDUALITY

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Why animal welfare?

Why should we care for animal welfare? Roughly speaking, all the possible answers can be categorized in two broad classes. First, we can care for animal welfare because it is good *for us* as humans. For example, an animal in a poor welfare condition is not as efficient as it could be if it were in good welfare. Sick sheep are likely to become an economic loss for the shepherd as sick guinea pigs are a source of bad data for the researcher experimenting on them. In these cases, animal welfare is purely instrumental to reach human goods (economic or scientific).

But animal welfare can be instrumental also for achieving human moral goods. Notoriously the well-known philosopher Immanuel Kant (1724-1804) argued against cruelty to animals because humans who are used to be cruel with animals are likely to behave unethically with human beings (Kant, 1997). Kantian duties towards animals are indirect: animals have no moral standing neither rights (they are just things, even if living). On the contrary, human beings have moral status and rights and we should not take the risk of threatening the human goods corrupting our character through indulgence in cruelty to animals. Kantian argument gives us some moral reasons to protect animal welfare, but they are ultimately rooted in the fact that animal welfare is good *for us*.

A second type of attitudes toward animal welfare is grounded on the idea that animal welfare is good / bad *for the animals*. A typical example of this attitude is the utilitarian argument for linking moral status to the capacity of experiencing pleasure or suffering. From the utilitarian point of view the reason of judging morally wrong a poor welfare condition is the fact that the animal has an interest in being well (Singer, 1993).

In this paper, I will argue in favour of two claims. First, I will defend the idea that only the second kind of concerns for animal welfare is genuine. More precisely, I will show that the concept of «welfare» makes sense only if we endorse the idea that it refers to a condition that is good or bad for the individual to which such a condition is attached. Second, I will argue that this subjectivistic logic about animal welfare can be of help when practically elaborating what animal welfare is and how it is recognized and protected. In other words, I will claim that animal welfare is properly understood only if the first person point of view of the animal is taken into account. Taking into account the animal's point of view can lead to a richer concept of animal welfare, where the satisfaction of species-specific needs can be merged with the fulfilment of the needs that animals have as a consequence of their individual and unique character and temper.

The moral logic of welfare

Imagine yourself as a kind of existence rather different than a human being. You are a highly valuable painting: Raffaello's fresco *The School of Athens*. One day, when, as usual, dozens of

visitors are passing in front of you, a man takes from his backpack a bottle and throws its content against you. Actually, the content was not water – as it could seem at a first sight – but acid. A part of you is terribly corrupted: the face of Plato is gone and also his famous finger pointing at the realm of ideas is burnt. If welfare is a concept just describing a condition of existence, then it can be said that you are really in a bad welfare.

But does this mental experiment make sense? Can you really imagine what is like to be Raffaello's *The School of Athens*? This experiment requires you to take the point of view of the fresco. But does a painting really have a point of view? Does a painting experience something when attacked by acid? This kind of mental experiment requires to break the boundaries of experience. Actually, we can take the point of view of painting just in a metaphorical sense.

Would it make sense to use the concept of welfare in this case? Or would it in the case of a broken glass? If we play attention to the way we normally manage the concept of welfare, then we realize that its proper uses are always referred to sentient beings, that is to beings that can somewhat «experience» the conditions to which we refer with the term «welfare». The *Oxford English Dictionary*, for example, defines «welfare» as «the health, happiness, and fortunes of a person or group». Welfare is always a condition *for* someone, not just *to* someone. A ruined painting is in bad state, but it is not in bad welfare. On the contrary, a sick chimp is in bad state and it is also in bad welfare. Her suffering and distress are bad in two main senses. First they are bad for the chimp herself, because if she could choose she would avoid them, just like everyone who puts by chance a hand on a hot surface pulls it away soon. Second, it can be bad *to us* if we are morally persuaded that suffering is morally bad, independently of who is the sufferer.

Incidentally, the example of a sick chimp can help us to clarify a point. In fact, we must distinguish the source of moral value from the reasons to attribute such kind of value. Actually, human beings are the source of moral value at all. World without human beings would be a place without morality (but maybe with some kind of proto-morality) (De Waal, 1996). It is our moral judgement that fills of moral value facts that otherwise would be valueless. But what are the reasons to project moral values on nude facts? One of particular importance (actually the most important) has to do with the suffering / pleasure of sentient beings. So, suffering is a condition that is bad for the sentient being because it is unpleasant and it is bad from the point of view of moral values. Distinguishing the source of moral value from the reason to evaluate allows to state that to be morally bad a suffering state must not necessarily be experienced by someone who is able to recognize it as *morally* bad.

Then, if a sentient beings is in bad conditions we have reasons to be morally concerned for him / her and these reasons are different from those we have when we are worried for non sentient objects. We would feel something if *The School of Athens* would be damaged by a fool. In example, we could be concerned because humankind has lost a source of aesthetical pleasure and reflection. We could feel really sad for this. We could morally blame the attacker for depriving future generations of the pleasure of appreciating the fresco. But, nobody would be sympathetic with the painting for some «suffering» caused by the acid. The moral concern for the fresco is not rooted in some experience of the painting. *Our* interests have been wronged off, not the painting's. For these reason, nobody could sensibly speak of this fact as an offence to the welfare of the painting.

When we speak of animal welfare we are using a concept that entails the idea that there are some conditions of animal life that are morally good or bad because there is animal who is experiencing them as good or bad. In other terms, when we use the concept of animal welfare we are endorsing, at least implicitly, the idea that non human animals have subjective experiences. Generally speaking, the moral logic of welfare is mentalistic as far as it is grounded, at least implicitly, on the idea that welfare is a state experienced by the animal.

Notoriously, the issue of animal minds is a hot topic for animal behaviour science. When N. Tinbergen sketched the famous four questions of ethology (Tinbergen, 1963), the issue of animal minds was excluded and still today the scientific reliability of researches on this topic is widely debated. Similarly, animal welfare science seems to be affected by general suspect about animal minds. If, from a general point of view, moral concern for animal welfare is grounded on the idea that animals matter because they have experiences (Duncan & Fraser, 1997), when it comes to studies about what animal welfare is and how it has to be promoted, the issue of animal minds become more problematic.

In fact, most of the animal welfare theories do not include indicators about animal mental states. Some theories are focused on biological functions, some look at behaviour and others take natural (wild) conditions of life as the basic parameter (Duncan & Fraser, 1997). Of course, none of these approaches *per se* denies that animals have mental states. On the contrary, most of their supporters are persuaded that through those indicators animal mental experiences will be safeguarded. Though, animal minds stay in the background, because they could undermine the scientific reliability of the theory. If the moral logic of animal welfare is deeply mentalistic, its scientific logic tries to avoid too much concern for animal minds. But a reconciliation of the two logics would be of great benefit for animal welfare science. Actually, being more positive about animal minds could lead to a richer conception of animal welfare and to more efficacious strategies for its protection and promotion.

Reading animal minds

Roughly speaking, the issue of animal minds can be summarized in two main questions: «Do non human animals have minds?» and «How animal minds can be read?». Of course, negative answers to the first question make the second one useless. Also, saying «no» to the second question would make meaningless the first one, for animal minds could exist but they would be unknowable. Anyway, here, I will make some remarks just on the second question, assuming a positive answer to the first one.

The possibility to know animal minds is a particular occurrence of the general philosophical problem about the knowledge of other minds (human minds as well). Strictly speaking, others are for us pure behaviour. We do not have direct access to other minds. I can see your face when you are eating your favourite chocolate cake. I see your lips smiling at each bite and I look at the shape of your eyes. I can also hear you munching, but I cannot directly know what you are feeling. From what I see I can just infer that what you are feeling is probably very similar to what I feel when I eat my favourite sweeties.

Without this kind of inferences, human life would be really puzzling. Imagine to be completely blind to other minds and to be unable, for example, to infer emotions from the other people's faces. Every social relation would be problematic, if not impossible. Luckily, human beings seem to possess a powerful device to attribute mental states to other human beings and also to anticipate their behaviour on the ground of attributed mental states. This capacity is usually labelled as *folk psychology* and it is the ability to explain and foresee others' behaviour by attributing mental states (Meini, 2001). By the same capacity we can attribute mental states to non-human beings.

Actually, attributing minds to other living beings is an unreflective procedure. Before asking if others really have minds, we think and behave like if they had. In fact, during the evolutionary process, this kind of capacity has been rewarded, i.e. for its utility in developing anti-predatory strategies (Mithen, 1996). Of course, the «innateness» of this capacity and its

evolutionary success do not grant for the truth of the attribution of mental states, as they could not exist at all.

Traditionally, attribution to non-human animals of human-like mental states has been labelled as a particular kind of mistake: *anthropomorphism*. The most radical charge of anthropomorphism that can be made is the attribution of mental states at all to non human-beings. As I have stated before, I am not interested here to argue about this topic and I assume that non-human animals have minds. So, the charge of anthropomorphism which I have to analyse is about the interpretation of animal minds not their existence. Animal minds are not denied, but they could be rather different from ours.

The way we interpret animal minds could be affected by a bias: our capacity of reading other minds is quite efficacious in the case of other humans, but when it comes to other species we cannot be sure of what other animals' experiences are like. I can know what you feel when you eat chocolate because when you are eating it you smile the same way I do when I am eating it. But how can I know what my dog feels when she eats the piece of chocolate fallen on the ground? Notoriously, this kind of scepticism has been brilliantly formulated in Thomas Nagel's question «What it is like to be a bat?» (Nagel, 1974). How can we be sure to be able to know mental experiences of organisms whose brains, nervous systems and sensory apparatuses are different from ours?

Actually, mental states have a qualitative nature that can be experienced just from the first person point of view (philosophy of mind labels this qualitative nature of mental states as *qualia*) (Tye, 2003). At present, we do not have machines allowing us to enter other minds and, for example, to be John Malkovich for a while. Does this mean that every possibility of knowledge of animal minds must be written off?

If we have good reason to be sceptical about what actually are animal experiences, we have also good scientific reasons to suppose that they should not be so deeply different from ours. At least, in the case of basic mental states (like primary emotions) we can suppose strong analogies between species evolved from a common ancestor. A principle of cladistic parsimony, for example, can favour in many cases anthropomorphic interpretations: «if two derived behaviors are homologous, then the hypothesis that they are produced by the same proximate mechanism is more parsimonious than the hypothesis that they are produced by different proximate mechanisms» (Sober, 2005). This principle grants a *prima facie* reliability of our folk attributions of human-like mental states to non-human animals on the ground of human-like behaviour.

Furthermore, mental states are private states but they are also the product of the biological functioning of the brain and the nervous system. Human mental activities have some biological correlates that are readable through, i.e., monitoring brain activity or blood sampling. The same happens to non-human animals. Identical or very similar results data from researches about these biological correlates can prove similar mental activities in humans and non-humans (Griffin, 1992)**.**

Merging folk psychology and scientific evidences, we are not blind towards animal minds. In fact, anthropomorphism can be converted from a mistake to a critical method of enquiry and research (Burghardt, 1991) Here, it is not my aim to further elaborate about this method, but what I want to stress is the possibility to include animal minds in scientific discourse. This inclusion is a step toward the reconciliation of the two logics of welfare, moral and scientific.

Animal welfare and animal individuality

As I have mentioned before, dominant approaches to animal welfare stress the importance of indicators like biological functions or typical behaviour. These approaches aim at elaborating lists of species-specific indicators that allow to monitor animal welfare conditions and to elaborate strategies to promote it. As far as these indicators are conceived to be reliable, they are objective, that is they are supposed to be valid for every particular individual of the species. This kind of objectivity is, of course, particularly useful to understand animal needs. But is it enough? Maybe there is something about welfare that goes lost. To look for the missing elements, we must turn back to the moral logic of welfare and to exercise a bit of «critical anthropomorphism».

As in the case of animals, human beings have some needs that are strictly related to their species. If we want to promote welfare of a particular human being, first we should be sure that these needs are satisfied. But if a human being had all of the basic needs satisfied, could we be sure that he/she would be in a good welfare conditions? For us, as humans, welfare is made also of something that exceeds the species-specific needs. There is an individual element in welfare. Human beings reach their good life through a series of activity that are not represented by the basic needs of the species. Through their life, human beings try to develop a particular and individual style of life that is determined by personal preferences and individual character.

Imagine of having all your basic needs satisfied but to be deprived of the opportunity to make choices about the person to date, what kind of movies to see, what kind of games to play and so on. Would you say that your life is really good or that you are really in good welfare? If decent survival conditions are satisfied, this does not imply that good welfare is reached. Promoting the development and flourishing of individuality is a huge part of promotion of human welfare.

My claim is that also animal welfare should include the promotion of individuality. But why should we look at animals not just as token of the same type (the species) that are numerically different but qualitative identical? In other words, why should we look at animals as individuals? The main reason is given by the fact that they are individual minds. If animal minds are not left in the background, it is easier to recognize that also animals have individual characters. Temperaments are shown by the way an animal experiences the environment, the relations with other co-specific and humans. Of course, individuals of different species will have different capacities of developing individuality, but here what I am interested in is just stressing the importance of individuality also for animal welfare.

Introducing the individual dimension in animal welfare raises two questions. The first concerns the methods to recognize the particular and different characters of individual animals. The second regards the strategies to promote the expression and development of individual characters and, so, of welfare in each of its components.

With regard to the first problem, useful hints come from methodologies aimed at qualitative observations of animal behaviour (Wemelsfelder *et al.,* 2000). For example, profiling made by naïve observers with folk terminology has been proved a reliable method to understand the individual character and «style» of animals. As F. Wemelsfelder has remarked, these methods focus on the animal as a whole rather than on separated pieces of behaviour (Wemelsfelder *et al.*, 2001). To perceive individual differences between animals, we should look at them as *agents*. This point of view cannot be gained if animal minds are denied or put in the background. To describe individual character, a mentalistic (and somewhat *folk*) terminology has to be used. How could we fully understand and manage the concepts of «shy» or «curious» without references to same mental state? Our capacity to sympathise with animals is grounded on the capacity to understand their minds.

The second question concerns the methods to satisfy individual needs. Given the individual nature of these needs, strategies must be elaborated case by case. Generally, it can be said that the promotion of welfare also in its individual dimension entails freedom as a necessary condition. The possibility for animals to express their character and to make their capacities flourish is increased by the possibility to be inquisitive and manipulative toward the physical and social environment. Elsewhere, I labelled this kind of life «Socratic» as far as it privileges inquiry and exploration over hedonistic pleasure (Pollo, 2004). On this basis, individual animal welfare is promoted where animals are given the possibility to choose and to satisfy their needs the way they like most.

Conclusion: two levels of animal welfare

I have tried to show that the moral logic of welfare is individualistic and mentalistic, while, the major trends in animal welfare science seem to leave animal minds on background. Reconciliation of the two logics can contribute to a richer understanding of animal welfare. Then, animal minds have to be considered a legitimate subject of scientific research. Objections to the possibility of «reading» animal minds are powerful but not conclusive and a critical use of anthropomorphism can help. In the end, folk psychology seems to work well and to be reliable.

Giving room to animal minds allows to recognize individual character differences among members of the same species. Looking at animals as individuals makes possible to elaborate a theoretical framework for animal welfare. Animal welfare is made of two levels. The first is constituted by species-specific needs that can be measured by objective lists and promoted through standard actions. The second is made by the individual needs beyond the basic needs shared by all the members of a species. If the moral logic of welfare is really understood, the second level of welfare ought never be forgotten.

Acknowledgments

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