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F. Mata

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A Framework for Using Epidemiology in Animal Welfare Science

F. Mata

CISAS – Centre for Research and Development in Agrifood Systems and Sustainability, Instituto Politécnico de Viana do Castelo, Viana do Castelo, Portugal

ABSTRACT
The potential advantages of using epidemiology in animal welfare research are substantial and are used with increased frequency. Collaboration between scientists of different fields, with different specific expertise is advantageous in the advancement of science. In this review, a framework to use epidemiology in animal welfare science is established. The different epidemiological study designs and analytical procedures are explored and put in an animal welfare scientific context. It is argued and demonstrated that epidemiology is used with advantage: in the identification of risk factors behind the development of maladaptation and abnormal behaviors; in the introduction of standardized procedures in research allowing comparisons between studies and facilitating the integration for evidence synthesis in systematic reviews and meta-analysis; by allowing animal welfare scientists to analyze complex settings such as farms or zoos. Mathematical modeling can also be used with advantage in risk assessment.

KEYWORDS
Animal welfare; case-control; cohort; cross-sectional; epidemiology

Introduction

The potential advantages of using epidemiology in animal welfare research are substantial (Green & Nicol, 2004), and have been used with increased frequency with farm animals (Collins, Asher, Summers, Diesel, & McGreevy, 2010). Collaboration between scientists of different fields, with different specific expertise is advantageous in the advancement of science (Hall, Feng, Moser, Stokols, & Taylor, 2008), and lack of expertise in the integration of different fields is identified as an issue (Houe, 2003).

It is therefore, important for scientists to gain knowledge in scientific fields different from those of their expertise and develop integrative skills to bridge disciplines.

It is the aim of this review to identify the potential for collaboration between epidemiologists and animal welfare scientists. A framework is established to inspire animal welfare scientists in the adoption of useful standard techniques, allowing the integration of animal welfare and epidemiology when advantageous. The different epidemiological study designs and analytical procedures are explored and put in an animal welfare scientific context. The bio statistical approaches used in the different study designs are also put into perspective. The illustration of this integrative framework is made using publications in the public domain, here provided for reference.

Collaboration in animal welfare science: Challenges and opportunities

Teamwork is an increasingly important attribute in science and research, and accordingly to Hall et al. (2008) has been privileged by research funders in recognition for its advantages in advancing knowledge.

CONTACT F. Mata ✉ fernandomata@ipvc.pt Rua da Escola Industrial e Comercial Nun'Alvares 34, Viana Do Castelo 4900-347, Portugal

Shen (2008) refers to the importance of interdisciplinary with its capacity to empower the acquisition of knowledge. Nash (2008) recognizes the increasing importance of individuals with this integrative capacity. The need for highly specialized individuals to advance the different branches of science creates a deficit of integrative capacity. The multi-skilled individuals are experts in integration and play a determinant role in teamwork. The understanding that integration is important in research is surging and creates in researchers, appetite for different disciplines (Houe, 2003).

Webster (2007) emphasized that animal welfare science is an area of overlap between disciplines: physiology, involved in measuring levels of cortisol to evaluate stress in order to assess welfare; ethology, involved in the study of normal/abnormal behaviors to evaluate husbandry practices, through the impact on the capacity of the animal to display a normal behavior; and veterinary science, involved in the study of health and suffering. Webster (2007) concludes that these disciplines working in isolation create gray areas, which fade away with their integration as animal welfare science.

Animal welfare also includes the evaluation of the animals' needs and their affective states, which emphasizes the importance of understand their behavior and therefore, cognitive capabilities (Nawroth et al., 2019).

Linking biostatistics, epidemiology and animal welfare science

Willeberg is probably one of the first scientists to relate animal welfare with veterinary epidemiology. He claims the primordial role played by veterinary epidemiology to assess animal welfare, and identification of risk factors for prevention of disease (Willeberg, 1997). The investigation of incidence, prevalence and risk factors, is part of the activity of qualifying and quantifying animal welfare issues.

During the 1980s surged the awareness that animal welfare could not be fully evaluated without considering the animals' health status (Broom, 1986, 1988). Disease is itself a measure of poor welfare but can be a confounder if not considered when evaluating other welfare issues. Is disease the cause of poor welfare itself or did it develop by any other reason? Willeberg (1991, 1993) raises the question and introduces epidemiology as an important tool in the identification of welfare risk factors.

Green and Nicol (2004) discussed advantages of using epidemiology in animal welfare science, and indicated it as the perfect tool in the identification of environmental, management and genetic risk factors with impact on animal welfare.

Millman, Johnson, O'Connor, and Zanella (2009) refers to the importance of collaboration between veterinary epidemiologists and animal welfare scientists. Between many other things, such as quantitative surveillance and study cause, origin and ecology of diseases, veterinary epidemiologists are trained to investigate the prevalence and incidence of disease in space and time. Veterinary epidemiologists identify risk factors and factors that may protect animals from poor welfare outcomes. Animal welfare scientists tend to investigate within a laboratory and/or controlled environment, capable of identifying human and environmental risk factors with impact on animal welfare (Edwards, 2007).

Veterinary epidemiologists are trained to perform experimental and observational studies at individual level and in more complex group-level settings (e.g., farms, parks) where interactions between risk factors exist. They use modeling techniques and risk analyses to assess the impact of different treatment or exposures. In complex settings, bias is limited by the ability to create study designs and use advanced analytic approaches considering factor interactions. Veterinary epidemiologists lack however, training in animal welfare and behavior measurement and evaluation, which justifies the collaboration between the two groups (Edwards, 2007; Millman et al., 2009).

Accordingly to Paton, Martin, and Fisher (2013) epidemiology has been used while studying the impacts of environment and infrastructure in animal welfare, which has been found advantageous in risk assessment in a variety of animal settings such as: fish farms (e.g., Turnbull et al., 2011), homes (e.g., Collins et al., 2010), farms (e.g., Dewey et al., 2009), zoos (e.g., Carlstead, Mench, Meehan, & Brown, 2013), and racing (Williams, Parrot, & Da Mata, 2012).

Biostatistics is a discipline with applications in several dimensions of the biological sciences, and as Curnow (1984) emphasizes, a biostatistician cannot anymore be a pure statistician, he needs to be a biologist as well, to be able to integrate both sciences. Biostatistics have a major contribution to epidemiology and had a major contribution in the scientific advances of biological sciences in general over the last 50 years (Breslow, 1996), specially by the combination with computing power (Gehan, 2000).

Researchers without expertise in design and data analysis should always involve a statistician in their research (or an epidemiologist once these have statistics expertise). Ideally from the beginning while planning.

A full range of statistical methods are used in epidemiology: general linear models, generalized linear models, survival analysis, meta-analysis, multivariate analysis, spatial analysis and others; sampling and power analysis are also techniques required for collection and preparation of data.

Nevertheless, we need to emphasize that animal welfare science is not limited and exhausted with the linkage between epidemiology, veterinary science and biostatistics. For example, the advances in the field of animal cognition has implications for animal management and practices. The physico-socio-cognitive processes and mechanisms have implications on human-animal interactions and in welfare ethics (Nawroth et al., 2019).

Applying epidemiology in animal welfare science

In epidemiology, a variety of research designs are used to plan the collection of data (Figure 1). Thrusfield and Christley (2018) considers a distinction between "modeling" and "studies". The different studies are then classified differentiating between "intervention" (researcher with an active role) and "observational" (passive role). Observational studies can be made in the present ("cross-sectional") or over time, which classifies them as "longitudinal". Longitudinal studies can then be made prospectively ("cohort" studies) or retrospectively ("case-control" studies). If the data is collected from groups of individuals, that is, a collective unit of analysis (e.g., farm, park, and herd), the study is named "ecologic". Some variability may be observed in this classification, function of different "classification axes" (Pearce, 2014). The majority of the studies have in fact mixed features (Silva, 1999).

Mathematical modeling

Mathematical modeling has been applied in biological sciences, and can be defined as the mathematical representation of quantitative events, to allow their predictions (Oliveira & Hilker, 2010). In epidemiology, the technique is used mainly to model patterns of disease occurrence, to allow the prediction of spatial and temporal spread, benefiting surveillance and the adoption of control strategies (Thrusfield & Christley, 2018). Motulsky (2004) is an example of a book introducing the use of linear and nonlinear regression to model biological data. Thornton (2010) and Mata (2014a) are good examples of applications.

Thornton (2010), while discussing future perspectives of animals in farms, identifies the inclusion of animal welfare parameters in best linear unbiased prediction (BLUP) animal models for estimation of breeding values. Several animal welfare issues have potential to be managed through selection, as they are heritable: e.g., canine hip dysplasia (Douglas, Mata, & Menem, 2015), feather pecking in poultry (Rodenburg, Buitenhuis, Ask, & Uitdehaag, 2003), tail bitting in pigs (Breuer et al., 2005).

Mata (2014a) developed a logit-log model to evaluation horse fitness for exercise through post-exercise heart rate recovery. It is argued that the model has a high degree of accuracy and the potential to be used in simple portable equipment allowing quick *in situ* evaluation of horse fitness.

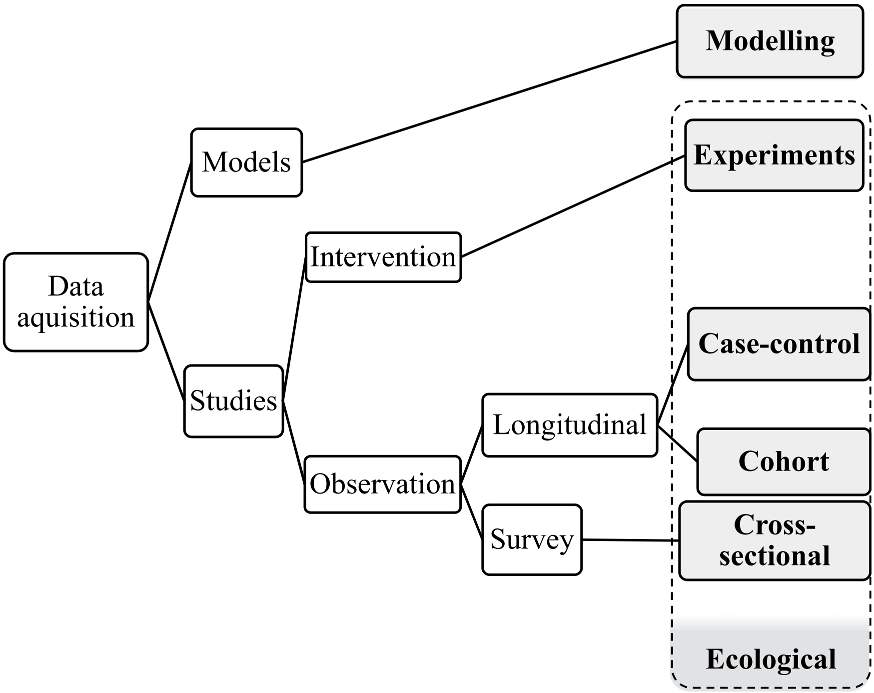


Figure 1. A possible classification of the different types of designs (shadowed boxes) to use in animal welfare epidemiology, with indication of the differentiating characteristics (clear boxes). Ecological studies are a particular type of studies with a collective unit of analysis rather than individual. Adapted from Thrusfield and Christley (2018).

Experimental studies

An experiment or a trial is a study made in controlled conditions. The investigator intentionally manipulates one or more “factors” of interest to evaluate their effects. These are measured between groups of individuals receiving or not a treatment related to a factor of interest. The “control” group is composed by individuals not receiving the treatment. This group may receive a “placebo” or sham treatment to avoid bias. Instead of a “placebo” group as control, we can have two or more treatment groups and in this case the different treatments are the controls of each other. The individuals in the different treatment groups should be similar with exception for the treatment they are subject to. The different treatments in the factor are named “levels” of the factor (e.g., Ruxton & Colegrave, 2011).

The allocation of treatments to individuals is done ideally “blind” to the eyes of the researcher, to avoid suggested or influenced readings of effects; this is not always possible as some factors do not allow that. For example, when studying the effect of a bedding material in poultry we can chose the different groups to allocate to the different beddings, but if the factor of interest is breed, the individuals cannot be allocated to other than their breed. In this example, we are obviously considering the individual as a flock or group of birds, and therefore the allocation of different beddings is done in different flocks. These aspects are used to classify the experiments as “randomized”, “semi-randomized” and “non-randomized”.

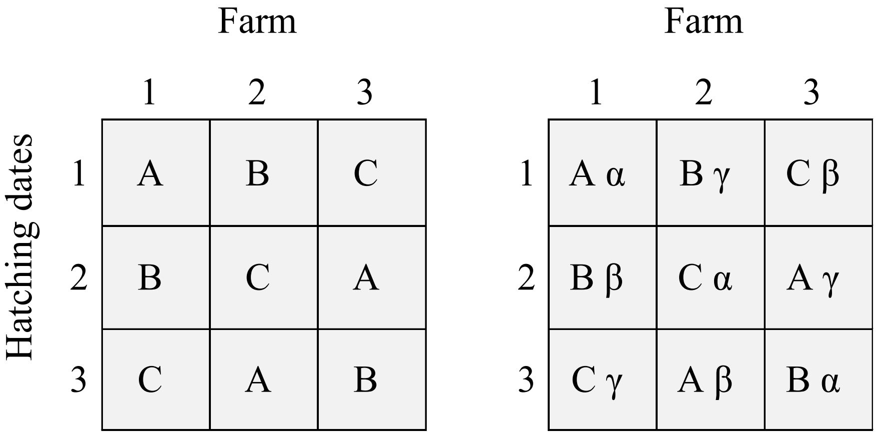
In randomized experiments, the individuals are allocated completely at random to the different treatment or treatments. In semi-randomized experiments there is more than one factor being analyzed; if one of the factors is not allocatable, different groups with equal number of individuals for each of the levels of that factor are created, and the second factor is randomly allocate within each of the groups. In non-randomized experiments, the factor is not allocatable. Using the previous example: in a randomized experiment one particular breed is used and randomly divided in three equal groups that are allocated to each of the bedding materials; in a semi-randomized experiment each of the three breeds is divided in three equal groups (nine groups of individuals) and the birds are allocated randomly within each of the beddings (the three breeds are represented in the three beddings); in a non-randomized experiment the three breeds are used in a particular bedding to test eventual differences between breeds (e.g., Ruxton & Colegrave, 2011).

The test of different effects can be done "within" the same subject or "between" subjects. Using again the same example, we may want to test the effect of the bedding material over time; different bedding materials have different properties (e.g., capacity to absorb humidity) and may convey different welfare measurements over time. If we include the factor time in our design with the levels 20, 40 and 60 days, we take "repeated" or "paired" measurements in the same individuals over time. In this example time is a "within subjects" measure and bedding is a "between subjects" measure. The factors tested in experiments are known as fixed factors when the measurements for the different levels are all present in the study, and as random factors when sampled. In the example used, bedding material is a fixed factor as we are testing exactly what we want, however for the factor time we have taken a sample of possible moments in time (we could have taken 18, 35 and 50 days or any other combination). Experimental designs include factors of interest, but sometimes nuisance factors need to be considered in the design. In a "full factorial" design, no nuisance factors are considered and all the combinations between the levels of the different factors being studied are present. In "blocked" designs, one factor of interest is considered together with one or more nuisance factors: the "split plot", the "Latin square", and the "Greco-Latin-square" designs include, respectively, one, two or three nuisance factors. Blocked designs contain all the combinations between levels of the factors, but do not contain replications for the nuisance factors; the number of levels of each of the factors is equal and the treatments are allocated at random. Nuisance factors refer to those factors not present in the study eventually capable of influencing the final results. Examples of nuisance factors are the interaction between the levels of the different factors not present in the study (if the study is not full factorial), or any other environmental factor not present at all in the study. In fact, in most of the experiments there are always nuisance factors difficult to control. We need however, to anticipate and control these as much as possible (e.g., Ruxton & Colegrave, 2011).

Blocked designs reduce the number of individuals in the experiment. They have, however the disadvantage of not allowing the study of interactions between factors. Using again the same example, if we are using three farms in the experiment and in each farm three hatching times, and if we use three different buildings with different ventilation systems in each farm, we may need to enter farm, hatching time and ventilation system as nuisance factors (Illustrated by Figure 2). A full range of other designs is found in the literature, based on the "combinatorics" theory of "orthogonal arrays" (e.g., Colbourn & Dinitz, 2010).

Experiments are statistically analyzed with ANOVA like models, which are included in the family of the "general linear models" (GLM). Parametric tests are used when the prerequisites are met: one, two or multiway ANOVAs, depending on the number of factors in the design; if the independence of the observations is not met, then repeated measures ANOVA should be used. There are alternative non-parametric approaches, namely the Kruskal-Wallis test for independent measures and the Freedman test for repeated measures. Ruxton and Colegrave (2011) and Vittinghoff, Glidden, Shiboski, and McCulloch (2012) are good examples of literature where the topic and the concepts exposed in this section are comprehensively introduced and explored.

Experiments should ideally be "complete" and "balanced", containing all the treatments and their combinations, and with the same number of observations per treatment. Occasionally animals are withdrawn, because they need to be moved, die or whatever other reason. If the number of observations is large, losing one animal does not have major implications and commonly the software treats this aspect by replacing a missing value with a mean value; however, if the number of observations is short or the number of withdrawals high, a type II ANOVA is preferred to the



Hatching dates	Farm 1	Farm 2	Farm 3
1	A	B	C
2	B	C	A
3	C	A	B

Hatching dates	Farm 1	Farm 2	Farm 3
1	A α	B γ	C β
2	B β	C α	A γ
3	C γ	A β	B α

Figure 2. In the Latin square deign (left) three different farms and three different hatching dates are eventual nuisance variables. In the Greco-Latin square design (right) ventilation system is a third eventual nuisance variable. Latin letters stand for three different bedding material and Greek letter for ventilation system.

traditional type I. There are three main types of ANOVA that differ in their mathematical process of calculating the sum of squares. Type II ANOVA applies more efficiently to unbalanced data (e.g., Langsrud, 2003).

So far, we have looked to statistical models dealing with dependent or response variables that measure something ("continuous", "interval" or "scale" data). Frequently the dependent variable is not continuous, e.g., count of the number of times a certain behavior was performed and existence or not of a certain behavior. In the first example, we have a "nominal" variable (count), in the second example we have a "dychotomic" variable (existence or not). These type of dependent variables are analyzed with a group of models known as "generalized linear models" (GzLM). These models use a "link function" to allow the response variable to vary linearly. With counts, we use link functions from the "Poisson" family (e.g., Poisson, "negative binomial", "log"), and with dychotomic variables we use link functions from the "binomial" family (e.g., "logit", "probit", "complementary log-log", "negative log-log"). These link functions are the most commonly used, however several other can be found in the literature (e.g., Hardin & Hilbe, 2012).

A GzLM is an extension of the GLM concept. Further extensions are the "generalized estimating equations" (GEE), a GzLM for random effects; and the "generalized mixed methods models" (GLMM), a GzLM for a mixture of random and fixed effects, also known as multilevel models. Further extensions and statistical approaches can be found in the literature (e.g., Myers, Montgomery, Vining, & Robinson, 2010).

St-Pierre (2007) made a review of the design and analysis of experiments using mixed methods in animal sciences. Trials can be very useful to test whatever aspect of husbandry in relation to welfare: e.g., bedding materials, housing designs, stocking rates, enrichments, etc. Examples of studies using a trial design are Mata and Mwakifuna (2012) and Mancera, Murray, Gao, Lisle, and Phillips (2014). Mata and Mwakifuna (2012) used a trial design to investigate mortality and predation in poultry. Mortality was modeled with production system and breed as factors. Predation was investigated in scavenging production systems only with breed as factor. The strength of the eggshell was used as covariate in both models. Death and predation were modeled with GzLM (complementary log-log link function). Mancera et al. (2014) while studying transport on eastern blue-tongued lizards used a Latin square design. The analysis of data was performed using GzLM (logit), GLM and non-parametric statistics (Kruskal-Wallis test).

Cross-sectional studies

Cross-sectional studies are surveys where a sample of individuals is selected within a defined population at a specific point in time. They are snapshots of the state of a particular population (species, breed, farming system, husbandry practice, subgroup, wild population, etc.) in a particular space (local, region, country, etc.) in relation to a particular variable of interest (presence or absence of disease, trauma, dysfunction, behavior, physiologic process, etc.), in a defined interval of time (Last, 2001; Thrusfield & Christley, 2018).

The first step in a cross-sectional study is the definition of the target population. This population can be a relatively small group of local individuals or a large global population. The inference promoted by the study is limited by the universe (population) considered. If a local sample is used, conclusions cannot be drawn for a wider population.

Power analysis for determination of sample sizes is the second step. With larger sample sizes, there is an increase in power analysis, which is also in dependency of the critical significance level considered; higher levels of significance require larger samples and have a higher power analysis. In the opposite direction is the variation of the data; larger standard deviation requires a larger sample size for the same level of significance. The power varies also with the type of test to be performed, and therefore with the type of data, being parametric tests more powerful than non-parametric. Hawkins, Gallacher, and Gammell (2013) makes a good review of the topic.

In the third step (sampling design), samples are ideally taken at random (probabilistically), ensuring the selection of a group of individuals, representative of the universe being targeted. Research constraints sometimes may determine non-probabilistic sampling methods; and convenience sampling is used to take advantage of circumstances allowing collection of data that could not be available at random (e.g., animals that are slaughtered or culled, may provide unique noninvasive opportunities to collect data otherwise difficult or impossible to collect). Non-probability sampling may be a source of bias and as consequence introduces limitations in the study. Probabilistic sampling should therefore be preferred; these include a variety of designs to allow the collection of representative groups of individuals: simple random, cluster, stratified, systematic, etc. A full explanation of methods, advantages and disadvantages of different probabilistic designs can be explored in the literature (e.g., Lohr, 2010).

After data collection, the last step is the analysis. Cross-sectional studies identify the presence or absence of variables of interest in individuals, allowing the study of relationships with particular characteristics of the individuals. These relationships are known as “risk factors”: incidence and prevalence of the variable of interest in relation to “exposure” or presence of characteristics in individuals (Pfeiffer, 2010).

The data collected is nominal, in the form of counts (e.g., number of animals with the variable of interest, exposed, or treated) or dychotomic (e.g., presence or absence of characteristic, dead or alive, male or female). Data can also be collected in other formats, such as scale (measurements) or nominal in the form of ranks. Nominal variables are analyzed with the recurrence to tables of contingency (Table 1), using chi square type statistics; using GzLM from the Poisson family (for counts) or binomial family (for dychotomic variables); or using non-parametric approaches in ranks, such as the Kruskal-Wallis with independent data and the Friedman test with repeated measures.

Examples of cross-sectional studies in animal welfare science are Ponzio, Busso, Ruiz, and Cuneo (2009), Mata (2015) and Mata, Johnson, and Bishop (2015). Ponzio et al. (2009) investigated the incidence of fur chewing in commercial Argentinian chinchilla farms. They conducted a survey and used a GzLM (logit) to analyses the data. Mata (2015) investigated risk factors (type of teeth and type of diet) and covariates (age) affecting the cats’ dentition health status. He used GEE to add for the repeated measures of teeth within the different subjects. Mata et al. (2015) investigated different types of bit injuries in ponies and horses used in sports. They used a GzLM (log) to model bone spurs, injuries in the commissure and tongue injuries in polo and races.

Table 1. The contingency table of observational studies and respective measures. A, B, C, D will represent the number of animals in each of the categories and N the total (Thrusfield & Christley, 2018).

	Condition present	Condition absent	Total
Postulated risk factor present (exposed animals)	A	B	A + B
Postulated risk factor absent (unexposed animals)	C	D	C + D
Total	A + C	B + D	A + B + C + D = N

In cross-sectional studies only N is predetermined
In cohort studies (A + B) and (C + D) are predetermined
In case-control studies (A + C) and (B + D) are predetermined

In cross-sectional studies
Prevalence odds ratio = A D / B C

In cohort studies
Probability condition present in exposed (Incidence for exposed) = A / (A + B)
Probability condition absent in exposed = B / (A + B)
Probability condition present for unexposed (Incidence for unexposed) = C / (C + D)
Probability condition absent for unexposed = D / (C + D)
Odds rate of condition, present, exposed (risk rate) = {A / (A + B)} / {B / (A + B)} = A / B
Odds rate of condition, present, unexposed = {C / (C + D)} / {D / (C + D)} = C / D
Condition odds ratio = (A / B) / (C / D) = A D / B C

In case-control studies
Probability of exposure with the condition = A / (B + D)
Probability of no exposure with the condition = C / (A + C)
Probability of exposure for controls = A / (B + D)
Probability of no exposure for controls = A / (B + D)
Odds rate of exposure (condition) present, exposed = {A / (A + C)} / {C / (A + C)} = A / C
Odds rate of exposure (control) absent, exposed = {B / (B + D)} / {D / (B + D)} = B / D
Exposure odds ratio = (A / C) / (B / D) = A D / B C

Cohort studies

Cohort studies are also known as prospective studies as from the perspective of the researcher they are set in the present to collect data in the future. In this type of study, the sample of the defined population is divided in segments (e.g., exposed, not exposed, and eventually considering different degrees of exposure) that are followed up to investigate the probability of development of conditions of interest. Normally, a large number of individuals is involved in the study, especially if the event of interest is rare. The period of time depends on the life cycle of the individuals and the characteristic of interest.

These studies allow the comparison of incidence and prevalence over certain periods or points in time. Incidence and prevalence refer to the proportion of individuals showing or developing a condition, respectively, at or during a particular time period (Thrusfield & Christley, 2018). Comparisons between segmented groups of individuals within the defined population can be established using odd ratios or risk ratios: e.g., incidence rate in males/incidence rate in females (Table 1 explains these concepts).

Again, we may be in presence of nominal, dychotomic and scale variables and the analytic models to be used are those mentioned in the previous sections. The novelty is the presence of the variable “time”, treated with a very specific type of model used in “survival” analysis.

Survival analysis introduces the concept of “censored” data. We may lose track of certain animals initially included in the study (lost to follow up in surgeries, sold by owners, owners moving, study ending before the event, etc.). If the event of interest still did not occur when the animal was last seen, we do not know exactly when it occurred or will occur, but we know that at that particular point in time (when it was last seen) did not occur. The time registered for such animals is measured up to the last time seen and is entered in the analysis censored. The models used in survival analysis allow censored animals to be included in the study, and we can therefore, make use of this incomplete but precious information. A good text book for reference is Klein, van Houwelingen, Ibrahim, and Scheike (2014)

If the event of interest is death the term survival is appropriate, but not otherwise (e.g. time to develop a condition). In any case, in time to event analysis, models are used to calculate the probabilities of the event taking place after a certain period of time. There are three main models to use depending on the data collected. If time is grouped (e.g., 0–6 month, >6–12 month, >12–18 month, >18–24 month, >24 month) "life tables" are used, otherwise we can use the Kaplan-Meyer or the Cox regression techniques. The Kaplan-Meyer technique is used for the analysis of factors of interest (e.g., breed, gender, exposure, housing system, etc.), allowing the identification of eventual significant differences in time to event probability for the different levels of that factor. The technique does not allow factorial design and therefore each factor is analyzed individually. Finally, the most flexible of the models is the Cox regression, allowing the inclusion of factorial designs and covariates (e.g., age at diagnosis, weight, height, etc.).

Examples of cohort studies in animal welfare sciences are Müller, Gaillard, Lackey, Hatt, and Clauss (2010) and Mata (2014b). Müller et al. (2010) while studying the effect of captivity on longevity of three different species of deer, used life tables. They used those results and compared them with data available on the life expectancy of the same species while in the wild. Mata (2014b) investigated survival to limb amputation in dogs diagnosed with appendicular cancer. The risk factors analyzed were behavior of the dog in the first week after amputation, gender, treatment type, age at diagnosis, castration, and type of cancer. A Cox regression was fit using dog behavior and type of treatment.

Case-control studies

Case-control studies are also known as retrospective studies as from the perspective of the researcher they are set in the present to collect data from the past. Individuals are chosen accordingly to the presence or not of a characteristic of interest (disease, trauma, dysfunction, behavior, treatment, etc.). Individuals with the characteristic are the cases and those without are the controls. The past of the individuals is then investigated to identify and differentiate degrees of exposure of postulated risk factors triggering the development of the characteristic of interest. The number of individuals to recruit to the study is smaller than in cohort studies, as the characteristic of interest, even if rare, is already diagnosed.

Examples of case-control studies in animal welfare sciences are Mata (2013) and Wylie, Collins, Verheyen, and Newton (2013). Mata (2013) produced a meta-analysis to evaluate the mastitis vaccination efficacy in dairy cows. In the different studies used in this meta-analysis cows developing the condition are the cases and those that do not are the controls. They are then retrospectively investigated for identification of vaccination or not, which allows the production of exposure odd ratios for determination of vaccine efficacy. Wylie et al. (2013) investigated risk factors (turnout, stabling, feeding, transport, exercise, farriery and health) for equine laminitis using a case-control study. After identifying the case group (horses with laminitis), the control group was built with a random sample taken from horses without the condition. Nominal variables were analyzed for association with Chi square type tests. Continuous variables were analyzed for significant differences of means by t-tests, or its non-parametric equivalent (Mann-Whitney U-test). A further analysis included a GzLM (logit) where the nominal variables entered as factors and the continuous as covariates.

Ecological studies

The previous studies have considered single individuals as the unit of analysis within a defined population; in ecological studies, the unit of analysis is collective, for example, farm, zoo, park, flock. Again, the universe of study need to be defined, e.g., farms in a region or country.

Ecological studies should be interpreted carefully, as the aggregated unit of analysis may introduce bias in the inferential process, leading to the so-called "ecological fallacy". The ecological fallacy is defined as “the bias that may occur because an association observed between variables on an aggregate level does not necessarily represent the association that exists at an individual level” (Porta, 2008, p. 51); Piantadosi, Byar, and Green (1988) discusses this in detail.

Ecological studies are used with advantage to identify geographical patterns of risk factors, and the ecological fallacy becomes an eventual problem only, when risk factors are associated with other geographic patterns, e.g., related with environment, sociology or demography (Wakefield, 2008). The most common type of ecological studies are cross-sectional, without dependency of time. Longitudinal ecological studies can, however, also be performed. The consideration made previously for observation studies in terms of analytical procedures apply, therefore, to ecological studies.

Examples of ecological studies are Mata, Williams, and Marks (2012) and Onyango, Mata, McCormick, and Chapman (2014).

Mata, Williams, and Marks (2012) in a case-control ecologic study, investigated risk factors (number of racers, distance of the race, conditions of the track and the number of fences) for the existence of pulled up horses in races (unit of analysis). They used a GzLM (logit) to model the probability of existence of pulled up horses and a GzLM (negative binomial) to model the number of pulled up horses.

Onyango et al. (2014) in a cross-sectional ecologic study, investigated risk factors (vaccination of ewes or/and lambs, presence of orphans, stocking rates, lambing season, weeds in the pasture and presence of disease in the flock) associated with orf disease in a sheep farm (unit of analysis). They used GzLM (complementary log-log) to model the probability of finding diseased lambs and GzLM (negative log-log) to model the probability of finding diseased ewes. The choice of models is always done after trying several and choosing the best fit.

Animal welfare implications and conclusion

Epidemiology can be used with advantage in the study of animal welfare (Collins, 2012; Collins et al., 2010; Collins & Part, 2013; Green & Nicol, 2004; Millman et al., 2009; Paton et al., 2013; Willeberg, 1991, 1997).

Epidemiology is useful in animal welfare in the identification of risk factors behind the development of maladaptation and abnormal behaviors. The source of these risk factors varies from genetic and environment to husbandry and management. For example, Mata and Mwakifuna (2012) found that highly selected chicken breeds, such as Rhode Island Red and Black Australorp show a maladaptation to scavenging production systems in the tropical and rural Malawi. Despite the excellent performance as layers in industrial production systems, “calcium depletion from the birds’ bones, limiting foraging and escaping ability may be the explanation, which ultimately increases susceptibility to disease and predation”. The hypothesis was raised after the establishment of a positive correlation of eggshell strength with predation and mortality. Eggshell strength is a trait positively selected in these breeds and may not be identified as a risk factor in industrial production systems, where designed rations are offered to the birds, but that is not the case in scavenging systems.

Epidemiology does not identify the underlying mechanisms associated with the problem. However, the identification that horses in races with snaffle bits are predisposed to significantly higher severity and prevalence of oral trauma than ponies in polo with gag bits (Mata et al., 2015), is highly valid even without an explanation for the reasons behind that fact. Sometimes a full explanation is not given but hypothesis are raised for further investigation as in Mata (2014b). While investigating causes leading to limb amputation in dogs, Mata (2014b) found that bitches have a higher association with cancer causes, while in dogs there is a higher association with traumatic causes. It is hypothesized that higher frequencies of mammary cancer in bitches and a higher degree aggressiveness in dogs, may explain the results.

Epidemiology has also the advantage of introducing the use of standardized procedures in research. This standardization as the potential to allow comparisons between studies and facilitates the integration for evidence synthesis in systematic reviews and meta-analysis. For example, mastitis is one of the most important welfare issues in dairy cattle and if some studies show a clear advantage in the use of vaccines, some others do not. Mata (2013) produced a meta-analysis of case-control trials of mastitis vaccines where a slight advantage in the use of the vaccine is made evident. It is concluded that vaccines however, must not replace all the other practices and biosecurity measures used to prevent the disease; vaccines can be used with advantage, but only as an extra preventive measure.

Epidemiology allows animal welfare scientists to leave the relatively small scale of the experiment in a laboratory environment. Complex setting such as farms or zoos may be difficult to replicate in a laboratory, and intervention studies are therefore more appropriate (Green & Nicol, 2004; Millman et al., 2009). For example, Mata and Lam (2013) identified risk factors associated with the feed, determining the helminthic burden of captive birds of prey. Other examples include collective issues such as stocking rates, or industrial equipment such as a ventilation system in farms, or welfare issues in horses during racing. Horses during races are pulled up due to injury, fatigue or lack of performance, which can raise welfare issues; Mata et al. (2012) used a retrospective case-control study to identify pulling up risk factors. Onyango et al. (2014), identified risk factors of orf disease in sheep, at farm and animal levels, using both farm and animal as units of analysis.

If most of the advantages of the use of epidemiology in animal welfare are related with the identification of risk factors, there is, however, an exception. Mathematical modeling can also be used with advantage in risk assessment (Collins & Part, 2013). Semantic models take a description of a system with identified risk factors as input and produce a weighted welfare score as output (Bracke, Edwards, Metz, Noordhuizen, & Algers, 2008; Collins, 2012; Paton et al., 2013). Expeditious models may be used *in situ* with advantage, in welfare assessment of the physical and affective state of the animals. For example, Mata (2014a) used a mathematic model to fit heart recovery data, allowing the evaluation of horse fitness for exercise.

Epidemiological studies applied to animal welfare science have limitations. Epidemiological data are correlational (Carlstead et al., 2013) therefore this kind of analysis cannot be used to infer causation and for the most part can only show associations between variables. However, it shows which of the factors evaluated are associated with positive or negative welfare outcomes. Carsltead (2013) also refers to eventual sources of error in the data collected difficult to correct and leading to data reduction.

Animal welfare is an interdisciplinary science. To more accurately evaluate animal welfare it is necessary to understand animals' behavioral and cognitive needs and capacities. Different fields of science can and should be brought together in collaborative work to empower the acquisition of knowledge. Epidemiology and biostatistics can be used wisely, when and where advantageous in animal welfare science.

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ORCID

F. Mata http://orcid.org/0000-0002-5687-7114

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