

Evaluation of Animal Welfare in Extensive and Intensive Dairy Farms and its Correlation to Infectious Diseases

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Research article

Keywords: animal welfare, farm animals, dairy cow, intensive housing system, extensive housing system, infectious diseases

Posted Date: February 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-185168/v1>

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Abstract

Background: The welfare of farm animals has become a growing concern in recent years. This is the first study that assesses dairy cow welfare by the application of an impartial, reproducible, functional check-list based on risk analysis which provides a numerical animal welfare index to each farm. We tested the effect of two different management conditions, housing with free access to pasture and indoor housing, on dairy cows kept in 36 farms and concurrently carried out the evaluation of different infectious diseases. Animal welfare assessment was performed in each farm through the CReNBA's check-list. Moreover, the prevalence of *Mycobacterium avium subsp. paratuberculosis*, *Chlamydia abortus*, *Neospora caninum*, *Bovine Herpesvirus* specific antibodies IgB and IgE and of *Bovine Viral Diarrhea virus* was tested in each farm through ELISA serological test. Unpaired t-Test was applied to assess differences among the two experimental groups; while, Kendall's Tau coefficient (T) was determined to investigate the relationship between studied variables.

Results: The result of each area provided an indication of the burden and importance of each of these on the final calculation of the animal welfare value. ELISA was positive with different mean percentages. The application of Unpaired t-Test showed that the intensive housing system group had higher percentages of *Bovine Herpesvirus* specific antibodies IgB compared to the extensive housing system group. The application of Kendall's Tau coefficient showed different correlations between studied areas and infectious diseases.

Conclusion: The hazard analysis areas assessed farming and management conditions of the farms, but these may have different effects as they are regulated by the animals' ability to adapt and are therefore less important when establishing the final welfare value. Those farms with the highest prevalence of infections could be associated with a poor level of business management. A proper evaluation of the problems encountered and a more accurate application of the check-list could prevent and control the spread of infections in farms. Our results want to be a contribution in breeding which provides farmers and veterinarians the instruments to improve animal welfare and farm business performance.

Background

In recent years, the interest in the evaluation of farm animals' welfare is growing [1]. Nowadays, animal welfare appears to be an essential component of the social pillar of sustainability for the dairy industry [2]. Veterinarians, trusted as medical experts, are increasingly being called on by the public and policy makers to be scientific and moral authorities in animal welfare issues [3].

Concern for animal welfare is nothing new; for their part, farmers have always been worried about the condition of their animals and they have to adapt their management practices in order to improve and optimize the herd's welfare [1, 4]. Throughout the world, dairy cattle are managed under a wide variety of management and housing systems and in different climates [5]. Good welfare is normally seen as the absence of illness or injury. More recently, the issue of animal welfare has been focused on pain or

distress that animals might experience as a result of widely accepted management practices, and the possibility that animals could suffer as a result of being kept under apparently “unnatural” conditions [4].

Animal welfare level varies considerably between dairy herds; variation among farms exists in management and housing conditions [6]. Animal welfare is a multidimensional concept, for this reason the improvement of the level in a farm by adjusting management or housing factors is complex [7]. The Welfare Quality® protocols for cattle aim to collect information concerning 12 criteria, divided into 4 essential principles of welfare: good feeding, good housing, good health and appropriate behaviour [6, 8]. Each principle must be interpreted on the basis of the special needs of animals of different species exposed to different systems of feeding, housing and management [9].

In the past half-century, animal production systems have undergone a radical transformation that has led to decrease the concentration of large herds in fewer specialized intensive farms, keeping animals in indoor housing system [10]. Housing cattle indoors year-round reduces labour inputs, facilitates the administration of high-energy diets and increases milk yield without growing farm size. Cows in indoor housing are also better protected against the adverse effects of extreme climatic conditions and endoparasites. Compared to pasture system, bedding surface can increase the prevalence of integument alterations [11].

For several reasons pasture-based dairy farming is often perceived as advantageous for animal welfare, particularly in comparison to intensive housing systems [7]. Cows on pasture sometimes experience a lower incidence of diseases such as mastitis and lameness [12]. Moreover, pasture can provide certain welfare benefits, in particular, cows have access to a more natural environment and they can perform behaviours that may be important to them such as grazing [7]. On the other hand, cows can also benefit from conditions provided indoors, in particular access to a high-quality diet and protection from extreme environmental conditions (e.g., heat, cold and wetness) [7, 12].

In nature, health disorders can be infectious or non-infectious. Different conditions or factors at the animals' level (age, parity, lactation stage, breed, immune status), as well as at the level of farming areas (housing, nutrition, climate, management) all together contribute to the occurrence of such disorders [13].

In 2011 the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) developed, by the Italian National Animal Welfare Reference Centre (CReNBA), a draft welfare assessment protocol and at first applied it in the dairy farms located in IZSLER's geographical competence area (Northern Italy). Later on, in 2012–2014, CReNBA's activities concerning dairy cow welfare assessment covered also biosecurity assessment and were extended to the entire Italian territory, thanks to the coordinated training of several veterinarians from different Italian Regions. This protocol is mainly based on findings provided by the European Food and Safety Authority (EFSA) publications, the Welfare Quality® assessment protocol for cattle and the draft regulation under discussion in Strasbourg (“Draft Revised Recommendations concerning Cattle”, revised version No 8, September 2009); minimum requirements provided by the law in force (Legislative Decree 146/2001, transposition of the Council

Directive 98/58/EC and Legislative Decree 126/2011, transposition of the Council Directive 2008/119/EC) are also taken into account [14].

Ragusa is the province with the highest zoo-technical vocation in Sicily (Italy); it boasts the presence of 1400 farms with a total of 80000 cattle, 30000 of which bred for milk production. For this research we worked with livestock farms and their productions in the province of Ragusa, considering the ethical and economic issues but mostly focusing on the health and hygiene aspects that are closely linked to animal welfare. Farm animal welfare has a close relationship with breeding conditions, farm management, animal health and hygienic characteristics of their products. The aim of this study was to assess the welfare level on dairy cows in the province of Ragusa, Italy, under two different management conditions: when providing free access to pasture versus when confining the animals to indoor housing. We concurrently carried out the evaluation of different infectious diseases.

Results

The total herd sizes assessed varied from 42 to 126 cows per farm, with a total of 1250 cows and an average of 73.53 cows, in Group 1; while they varied from 28 to 418 cows per farm, with a total of 2831 cows and an average of 149 cows, in Group 2.

Figure 1 shows the different mean percentages of animal welfare assessment we have found.

The results of serological tests analysed through ELISA indicated that cows were positive with different mean percentages in each housing system, as shown in Fig. 2.

The application of Unpaired t-Test showed that Group 2 had higher percentages of *Bovine Herpesvirus* specific antibodies IgB compared to Group 1 (Table 1).

Table 1

Mean values and standard deviations (\pm SD) of *Mycobacterium avium* subsp. *paratuberculosis*, *Chlamydiophila abortus*, *Neospora caninum*, Bovine Herpesvirus specific antibodies IgB and IgE and Bovine Viral Diarrhea virus expressed in percentage obtained in both groups along with statistical results of Unpaired t-Test.

	Group 1	Group 2	P	t	df
<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>	2,94 \pm 0,03	5,04 \pm 0,06	0,1973	1.315	34
<i>Chlamydiophila abortus</i>	1,41 \pm 0,02	1,18 \pm 0,03	0,8017	0.2531	34
<i>Neospora caninum</i>	15,61 \pm 0,11	25,01 \pm 0,22	0,1204	1.593	34
Bovine Herpesvirus specific antibodies IgB	31,02 \pm 0,30	60,83 \pm 0,39	0,0154	2.551	34
Bovine Herpesvirus specific antibodies IgE	15,41 \pm 0,21	20,18 \pm 0,32	0,6021	0.5263	34
Bovine Viral Diarrhea virus	39,36 \pm 0,33	50,13 \pm 0,39	0,3831	0.8835	34

The application of Kendall's Tau coefficient showed a significantly positive correlation between Area A and *Bovine Herpesvirus* specific antibodies IgB ($T = 0.4159$), Area A and *Bovine Viral Diarrhea virus* ($T = 0.3754$), Area E and *Bovine Herpesvirus* specific antibodies IgB ($T = 0.3435$) in Group 2. On the other hand, it showed a significantly negative correlation between Area E and *Chlamydiophila abortus* ($T = -0.4621$) in Group 1, Area C and *Neospora caninum* ($T = -0.4269$), Area D and *Neospora caninum* ($T = -0.4904$) in Group 2.

Discussion

Consumers (influencing processors and retailers) have long-term interests in the security and quality of milk products. Moreover, more recently, the interest in housing and care of dairy cows and their associated products has been increased [4, 15, 16]. Management practices and housing system have been reported to commonly influence animal profitability, health, milk quality, reproduction and well-being, as well as farm productivity [17, 18].

The check-list we have used in this study represents a functional, reproducible, impartial and smart instrument based on risk analysis which permits to assign a numerical animal welfare index to each farm and also, by the data collected in each area, to provide veterinarians and breeders with the tools useful to improve farm management and structures, respecting farm sustainability. The evaluation of animal welfare data resulted with different percentages in the single areas and in the overall animal welfare scores, in general with values slightly higher in Group 2. In particular, Area A, Area B and Area E percentages, which analyse managerial and structural factors, resulted higher in Group 2 respect to Group 1; while the percentages of Area C, which analyses "Animal based measures", resulted slightly higher in

Group 1 respect to Group 2. Actually, the subject's ability to adapt to the environment lies between its living conditions and welfare. As a consequence, for a proper evaluation it is important to take into account not only the housing facilities, but also the effects of these on the animal. The cows showing discomfort present physical signals that can be observed, interpreted and evaluated. Such discomfort is frequently linked to pathological conditions (lameness, mastitis, skin alopecia), to abnormal behaviours (fear, aggressiveness) or to alterations in physiological conditions (body condition). These situations can be pointed out through animal-based measures in order to detect both health and non-health problems that affect the animals not living in conditions of welfare. The partial result of each area also provides an indication of the burden and importance of each of these on the final calculation of the animal welfare value. Based on the obtained results, the studied animal welfare scores seem to give only limited information about the welfare level of the herd. Nonetheless, our results indicated that cows bred with an intensive housing system had slightly better scores, these results were in discordance with previous findings establishing that intensive housing systems could be associated with many behavioural and welfare problems, in contrast to pasture-based systems, where regular outdoor exercise seems to have positive effects on health and welfare of dairy cows [10, 19, 20, 21]. Continuously housed systems are perceived to offer less behavioural freedom than pasture-based systems and as such interpreted as offering less welfare [19]. Looking for "normal" cattle behaviour, Kilgour [22] identified and reviewed 22 such studies. Cattle have a wide behavioural range, which include 40 identifiable categories. Grazing was the most common behaviour, followed by ruminating and resting, with these three categories accounting for 90–95% of an animal's day. These data revealed most grazing is performed during the hours of daylight, with little grazing at night; moreover, cattle spent more time resting and ruminating at night. Furthermore, there is a diurnal rhythm of behaviour, characterized by peaks of grazing activity associated with sunrise and sunset. Few studies have compared dairy cow behaviour in pasture vs continuously housed production systems. Animal welfare is a multi-criteria characteristic [19, 23].

The negative correlation between *Chlamydiophila abortus* and Area E leads us to think that a better level of biosecurity decreases the spread of this disease. This is in accordance with Anstey et al. [24] who stated that animal husbandry and management systems can potentially influence infection loads in cattle. According to Cascone et al. [25], the negative correlation between *Neospora caninum* and both Area C and D in Group 2 showed that increasing control in farms with intensive housing system positively affected the prevalence of infection. It is very difficult to eradicate this disease because dogs can be found wandering around farms having a close contact with cattle. Therefore, the presence of dogs on a farm could be a potential risk to provide the increasing chance of horizontal transmission [26].

Endemic diseases such as Bovine Herpesvirus and Bovine Viral Diarrhea virus can be transmitted from a farm to another if protections are not adequate [4, 15, 27]. Our investigations have shown that Bovine Herpesvirus and Bovine Viral Diarrhea virus infections are present in higher number in herds with intensive housing system than those with extensive housing system. Moreover we found a positive correlation between Area A and both *Bovine Herpesvirus* specific antibodies IgB and *Bovine Viral Diarrhea virus*, and between Area E and *Bovine Herpesvirus* specific antibodies IgB in Group 2. These results are in contrast with Blokhuis et al. [28] who stated that improving animal welfare can positively affect

numerous aspects of product quality and disease resistance; these effects have direct relevance to food quality and safety. It was also shown that in the absence of control, prevalence of Bovine Herpesvirus is typically high both at animal and herd levels [29]. The quality of stockpersonship affects the welfare of animals in the performance of routine tasks such as feeding, cleaning, etc. Assessment of this relationship underlines the importance of stockpersonship in animal welfare. Negative behaviour and sometimes handling of animals could induce stress and cause injury to animals [30]. Prophylactic measures such as routine diagnosis, reproductive control and rigorous health care such as cleaning of facilities, avoiding contact with neighbouring herds, acquiring animals with a negative diagnosis and using an artificial insemination program should be recommended and implemented in the properties with the aim of reducing reproductive losses caused by these infections.

Conclusions

The final outcome of the assessment system application is that to assign a standardized evaluation index to assess animal welfare on each farm, and also, by the data collected in each area, to provide veterinarians and breeders with the tools to improve farm management and structures, respecting farm sustainability. The partial result of each individual area, on the other hand, provided an indication of the weight and importance that each of them has in the final composition of the value of animal welfare. The index was obtained by adding up the assessments deriving from the responses for each single item and weighed in relation to the importance that each of these had in defining the state of welfare. In this study, the welfare of dairy cows farmed in intensive housing systems was found to be poorer. Several factors, whose assessment and measurement, however, are still an open research topic, are judged to have considerable impact on dairy cow behaviour. Only very weak associations were found between the selected infectious diseases. These extremely weak results may have been affected by the complexity of animal and environmental factors that can influence the animal welfare assessment. The farms in which there is a higher prevalence of infections could be associated with a poor level of business management and, probably, with an underestimation of the problem. A more accurate application of the checklist can help farmers prevent and control the spread of the infections. Clearly, risks to the dairy industry can be reduced if dairy farmers positively respond to some changes, for instance, by increasing their efforts to increase biosecurity, while concurrently providing assurances of high standards of animal welfare. The hazard analysis areas assess farming and management conditions of the farms, but these may have different effects as they are regulated by the animal's ability to adapt and are therefore less important when establishing the final welfare value.

Methods

The study was carried out from January 2017 to December 2018, and was performed in 36 dairy farms located in the province of Ragusa, Italy (36°55'48" N, 14°44'24" E and 515 m above sea level), where the climate is warm and temperate. A total of 4081 cows were enrolled in this study. The examined farms

had a variable consistency of lactating cows belonging to the following breeds: Italian Friesian, Italian Brown, Red Pied Fleckvieh, Jersey and crossbreeds. The age ranged from 6 months to 12 years.

Farms Management

The farms were divided into two groups on the basis of different managements: Group 1, represented by 17 farms, had an extensive housing system; Group 2, represented by 19 farms, had an intensive housing system.

Group 1 cows were kept in a grazing area of about 5–7 ha, at least 10 hours a day. In these grazing areas, there are herbs typical of the Ragusa plateau, Carob trees, which act also as shelter, and large pools of water. These areas are bordered by stone walls about one and a half meters high, built with an ancient technique. The cows spent the rest of the day in an area with a barn that serves as a refuge (from heat in summer and cold in winter). These barns are generally close to the milking parlour. Artificial insemination was practiced in some farms while in others natural fertilization with bull was performed. They were fed from May to September with 10 kg of fodder, 15 kg of hay (vetch, oats and barley) and 15 kg of silage (corn or silo grass) on average; while, from October to April, they were fed with the same diet, except silage as the season allows a lush pasture. Group 2 cows were kept in a stable with a surface area providing between 6 and 7 m²/head or as many usable cubicles according to the number of animals. They were fed with 8 kg of fodder, 14 kg of hay (vetch, oats and barley) and 23 kg of silage (corn or silo grass) on average. Each farm then had different supplements of 1 to 2 kg (soy, beet, sunflower, cotton, alfalfa). In both groups, water was available *ad libitum* and the milking routine included pre and post dipping.

The average daily milk production was 27 litres in Group 1 and 32 litres in Group 2. In bulk tank milk the average milk fat composition was 3.68% in Group 1 and 3.60% in Group 2, and the value of milk proteins was 3.50% in Group 1 and 3.40% in Group 2.

The protocol of this study was reviewed and approved in accordance with the standards recommended by the *Guide for the Care and Use of Laboratory Animals* and the Directive 2010/63/EU. This study did not involve experimental animals. The animals did not suffer as it was performed a single blood sample in the caudal vein and the compilation of a check list was based on the visual observation of the animals. The research was performed as part of the research project with grant number IZSSI RC 12/15 approved by the Ministry of Health. Informed consent was obtained from all the farmers involved, who joined the project voluntarily.

Animal Welfare Assessment

The method we used in this study is based on the analysis of two data groups: the first group refers to the assessment of the hazards occurring as a result of environmental conditions (facilities, equipment,

management and microclimatic conditions); the second group refers to the assessment of the risks, with the concerned adverse effects (health consequences). Hazard assessment is performed using parameters divided into five areas, respectively: Area A - "Farm management and personnel"; Area B - "Facilities and equipment"; Area C - "Animal-based measures", for carrying out the assessment of the risk and of the consequent negative effects on cattle; Area D - "Inspection of microclimatic environmental conditions and alarm systems", in the event of serious negative events (e.g. fire); Area E - "Biosecurity", to assess the level of prevention against the introduction and/or spread of infectious diseases in the cattle-shed.

The check-list used consists of 90 items both for extensive and intensive housing systems. Each item has three (negative, acceptable, positive) or two (negative and positive) choice options. Not all the inspection items have the same weight in determining the final welfare score: some assessments have a multiplication or division algorithm that increases or reduces the importance when determining the final welfare score. This protocol was applied by a trained veterinarian on each farm and each check-list, filled in all its parts, was placed on-line on the appropriate site created at the portal of the CReNBA, which issued a certificate of "Animal Welfare and Biosecurity Assessment" by assigning a score for each of the parameters and an overall score to each farm. The set of these evaluations was subsequently entered into an algorithm that returns a value expressed as a percentage (on a scale from 0 to 100), able to identify the general welfare conditions of the herd. The final calculation of the scores in the various areas and those of the overall welfare and biosecurity was not carried out by the assessor, but by a specific CReNBA software, available through the website <http://benessereruminanti.izsler.it>. After the final aggregation of measures an overall score was reached to assign the assessed farm to one of the four general welfare categories ("not classified", "acceptable", "enhanced" and "excellent"). Each assessment took 2–3 hours and was carried out around two hours after milking, between 10 and 11 am. Milking took approximately two hours in each farm. All farms used milking machines.

Blood Sample Collection

Blood samples from all animals present in the examined farms were collected after animal welfare assessments by coccygeal venipuncture. They were put into vacuum tubes (Vacuette™, Greiner Bio-One) with no anticoagulant additive and centrifuged at 3500 rpm for 10 minutes. The obtained sera were transferred into plastic tubes. These were analysed for the detection of *Mycobacterium avium subsp. paratuberculosis*, *Chlamydiophila abortus*, *Neospora caninum*, *Bovine Herpesvirus* specific antibodies IgB and IgE, *Bovine Viral Diarrhea virus* antibodies, using an indirect Enzyme Linked Immunosorbent Assay (ELISA), as per the manufacturer's instructions (ID.Vet, Grabels, France). Each serum sample was tested in duplicate and the final results were read by a spectrophotometer, measuring the optical density (OD) at 450 nm.

Data Analysis

The data collected from the check-list drawn up by the CReNBA and the laboratory assays were entered and stored in a Microsoft Excel spreadsheet, screened for proper coding and errors, and analysis was done.

The obtained data were expressed as Mean \pm Standard Deviation (SD). They were analysed for normality by Shapiro-Wilk test and for homoscedasticity by Bartlett test. Unpaired t-Test was applied to assess differences in the studied parameters between the two experimental groups. P values < 0.05 were considered statistically significant. Moreover, Kendall's Tau coefficient (T) was determined to investigate the relationship between the areas and the studied infectious diseases. Statistical analysis was performed using the STATISTICA software package (STATISTICA 7 Stat Software Inc., Tulsa, Oklahoma).

Abbreviations

IZSLER: Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna; CReNBA: Italian National Animal Welfare Reference Centre; EFSA: European Food and Safety Authority; ELISA: Enzyme Linked Immunosorbent Assay; OD: optical density; SD: Standard Deviation.

Declarations

Ethics approval and consent to participate: The protocol of this study was reviewed and approved in accordance with the standards recommended by the *Guide for the Care and Use of Laboratory Animals* and the Directive 2010/63/EU. This study did not involve experimental animals. The animals did not suffer as it was performed a single blood sample in the caudal vein and the compilation of a check list was based on the visual observation of the animals. The research was performed as part of the research project with grant number IZSSI RC 12/15 approved by the Ministry of Health. Informed consent was obtained from all the farmers involved, who joined the project voluntarily.

Consent for publication: Not Applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: Authors have no competing interests.

Funding: This research was funded by the Italian Ministry of Health; grant number IZSSI RC 12/15. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions: Conceptualization F.L. and G.C.; methodology F.A.; software L.P. and C.G.; validation G.P., V.M. and R.S.; formal analysis L.P. and C.G.; investigation F.L. and G.C.; resources G.C. and V.M.; data curation F.L. and L.P.; writing—original draft preparation L.P.; writing—review and editing L.P., G.P. and C.G.;

visualization F.A. and R.S.; supervision G.P.; project administration G.C.; funding acquisition V.M. and G.C. All authors have read and agreed to the published version of the manuscript.

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Acknowledgements: Not Applicable.

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Figures

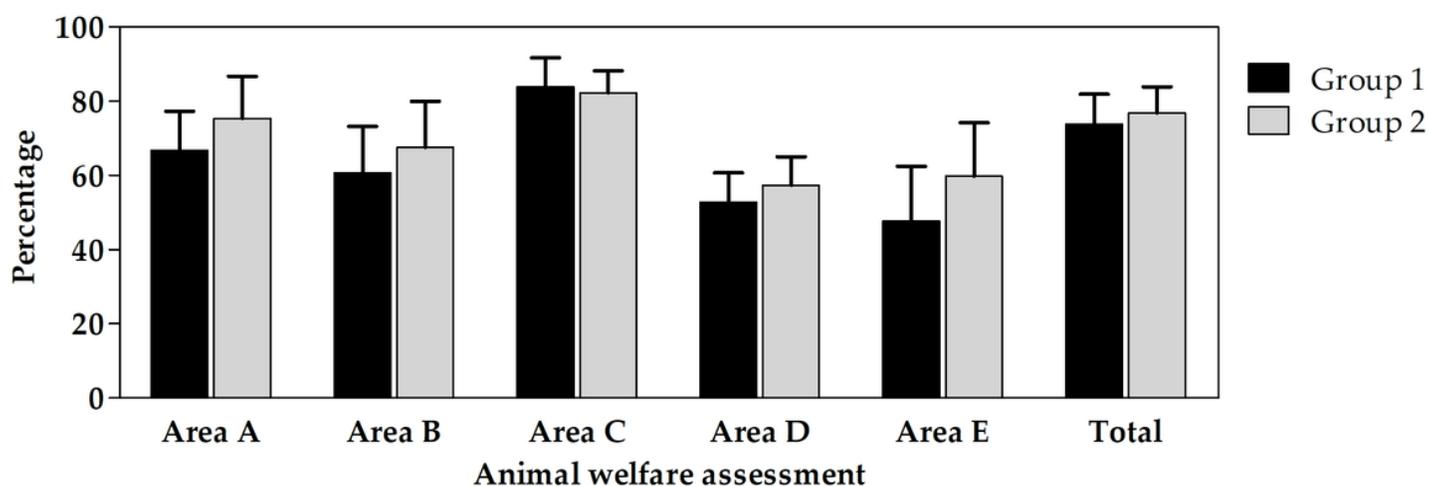


Figure 1

Mean \pm standard deviations (\pm SD) of animal welfare assessment Areas (Area A, B, C, D and E) in Group 1 (17 farms with an extensive housing system) and Group 2 (19 farms with an intensive housing system).

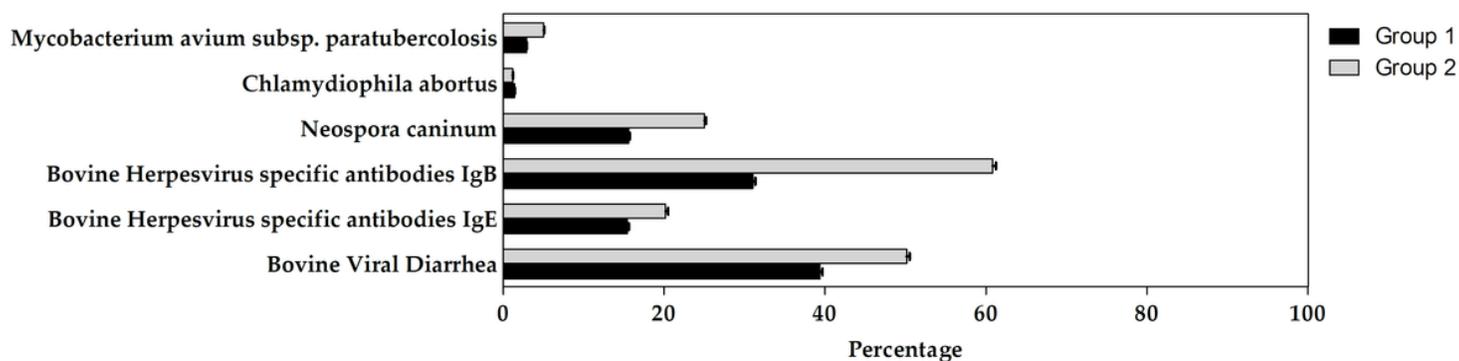


Figure 2

Mean \pm standard deviations (\pm SD) of Mycobacterium avium subsp. paratuberculosis, Chlamydia abortus, Neospora caninum, Bovine Herpesvirus specific antibodies IgB and IgE, Bovine Viral Diarrhea virus in Group 1 (cows bred with extensive housing system) and Group 2 (cows bred with intensive housing system).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Animalwelfarechecklist.pdf](#)