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Reproductive assessment

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Keywords: oestrus, vocalisation recorders, ultrasound sonography

Introduction

There has been an increasing trend in herd size in a number of countries which is associated with an increase in the number of cows per stock person. More specifically in the dairy cattle sector in Europe, since milk quota removal by EU in April 2015, many countries have implemented expansion programs mainly in the form of increasing cow milk yield and/or cow number [1]. This expansion could result in less time and attention dedicated to each cow, which may eventually impact the health and reproductive status of the herd. Therefore, tools which aid reproductive management, especially those that are automated, are particularly valuable.

Oestrus, commonly referred to as 'heat', is a behavioural sign and strategy to ensure that the female is mated close to the time of ovulation. Oestrus is an external and visible sign of ovulation, which is an internal and invisible event [2]. The efficiency of heat detection (HD) can be defined as the percentage of eligible cows seen or detected in heat. Heat detection is vital for synchronisation protocols as well as breeding programs, more specifically artificial insemination and embryo transfer programs. Poor HD is costly and proper heat detection needs to be acknowledged as the critical component of reproductive management. There are a number of methods, techniques and tools currently available and being implemented for HD, some of which can be combined to improve the HD efficiency and accuracy. Visual observation involves a trained observer recognising and recording signs of heat – with standing heat as the main behavioural sign of the oestrus phase [3]. The visual HD method is still recognised as the ‘gold standard’ for validating a newly established system, albeit laborious and time consuming. Electronic devices, particularly activity systems, have been widely introduced by different manufacturers in order to reduce the degree of dependence on visual HD [4]. Some of these systems have shown improvements in terms of efficiency, accuracy, and, more importantly, labour costs over visual observation [5]. There are, for example, pedometers attached to the leg of cattle, which detect an increase in the number of steps taken per hour during oestrus [1]. Recently developed pedometers are able to not only detect activity of cattle, but also the lying time and body temperature of the animal, thus increasing the specificity and sensitivity rate [6]. Despite this improvement in specificity and sensitivity, almost all cattle units use these HD tools to aid but not replace the visual observation method.

Rectal palpation of the reproductive tract and ultrasound scanning are commonly used methods to directly detect pregnancy in cattle [7], [8], [9]. Fricke [10] stated that an early pregnancy detection method must fulfil several criteria such as: **a)** high sensitivity (i.e. correctly identify pregnant animals); **b)** high specificity (i.e. correctly identify non-pregnant animals); **c)** inexpensive to carry out; **d)** simple to conduct under field conditions and **e)** an ability to determine pregnancy status at the time the test is performed. Rectal palpation is one of the oldest and still widely used techniques for pregnancy detection in cattle. Early pregnancy can be diagnosed via palpation of the amniotic vesicle or slipping of the chorioallantoic membranes between the thumb and forefinger. Despite the great risk of pregnancy loss as a result of rectal palpation, this method remains the ‘reference’ with whitch newly established direct methods will be compared prior implementation in the farm [10]. In comparison, transrectal ultrasonography is often the preferred choice of cattle practitioners, as this method is not directly associated with complications to the pregnancy [11] as well as being less intrusive than rectal palpation [12], [13]. Although attempts have been made to detect pregnancy in cattle at an earlier stage via ultrasound [14], [15], [16], pregnancy diagnosis should not be confirmed before a ‘rapid and reliable detection of an embryo with heartbeat’ is possible, in order to improve accuracy and avoid producing false positives [10].

Heat detection

Prerequisites

This guideline for HD in cattle in experimental units should be applied for both visual and automatic methods. A comprehensive guideline on managing and recording HD has been provided by the International Committee for Animal Recording (ICAR; https://www.icar.org/index.php/icar-recording-guidelines/). The Animal Trait Ontology for Livestock (ATOL) numbers linked with this guideline are: **ATOL_0001713, ATOL_0001789, ATOL_0001717, ATOL_0000992,** and **ATOL_0000993** (for complete list of ATOL please visit https://www.atol-ontology.com/en/erter-2/).

A – Visual (manual) heat detection

1. In addition to the primary sign of standing heat (standing and allowing others to mount), secondary heat signs can include roughened hair at the tail-head, sniffing, nuzzling and licking the ano-genital region of other animals, milk yield decline, and vulval mucus discharge, and should be noted by the observer. It is recommended that the HD operator records the signs observed [17].
2. Animals should be allowed to interact freely, particularly during the evening and early morning when most of the ‘heat’-associated primary and secondary signs occur.
3. Observation of animals for HD should be carried out for 2–4 per d, particularly where there is not an automatic HD tool available in the unit.
4. In intensive housing systems, animals should be allowed to interact freely for approximately 30–40 min to observe potential heat signs.
5. Heat detection **should not** be completed during the feeding time.
6. During days with high temperature/humidity index (THI>75) HD should mostly be carried out during the evening.
7. The area where animals will interact should not impede an animal's movement (e.g. slippery or muddy).
8. Hooves should be trimmed and any possible infections of the legs should receive immediate treatment in order to remove any potential obstacle to movement, which could result in cows not displaying heat signs.
9. One staff member of the unit should be assigned for HD. This staff member should be trained properly by an experienced heat detector technician.

B – Automatic heat detection systems and data validation

1. Farm staff should be aware of the ovulation period, which occurs 29–33 h after the onset of increased physical activity and 17–19 h after increased activity ends in dairy cattle [18], for optimal artificial insemination.
2. The sensitivity of the automatic HD systems must be compared with visual oestrus detection, to ensure system accuracy.
3. The HD operator must be aware of the milk yield level and profile. Herd parity and environmental temperature are greatly associated with the animal's physical activity, which in turn will influence the sensitivity (i.e. correctly identify animals in oestrus) of the HD tool. These parameters should be carefully monitored.
4. The research unit needs to clearly describe the gold standard (reference) method utilized for a ‘true oestrus period’, particularly regarding the false positive and negative data generated by the system.
5. The activity threshold which generates an oestrus alerts for animals on the HD system should be clearly stated.
6. Where pedometers are used as a HD tool on a pasture-based system, the specificity (i.e. correctly identifying animals not in oestrus) of the tool must be considered due to the movement of the animals during pasture rotation and the impact this may have.
7. Animal-mounted sensors, as well as the transponders, receivers and the antenna of the HD

system, must be checked every 4–6 months to ensure they are functioning correctly.

8. Where the HD tool generates an oestrus alert for a particular animal, the operator should confirm the alert by visually observing the heat signs.

Heat detection via vocalisation

Prerequisites

This guideline details the recording of vocalisations in ruminants and the assignation of vocalisations to individuals in group-housed ruminants. Although vocal behaviour is rarely used in studies of ruminants there is evidence that it can indicate physiological conditions such as oestrus or relate to stress and pain. In order to assess vocalisations and then assign them to an individual, the current gold standard method uses audio-visual recordings. The Animal Trait Ontology (ATOL) and Environment Ontology (EOL) numbers linked with this guideline are: **ATOL_0000372, ATOL_0000945, EOL_0001903** and **EOL_000036** (for complete list of ATOL please visit https://www.atol-ontology.com/en/erter-2/).

A – Monitoring vocal behaviour by audio-visual recordings

Animals should be housed in such a way that all individuals are clearly visible from the camera perspectives. It is recommended that more than one camera is used to record the experimental group in case an animal is obstructed from view from one perspective while another is necessary to assign vocalisations to a certain individual. Therefore it should be noted that large groups and large observational areas can produce more errors during assignment of vocalisations.

1. Cameras should be positioned in such a way that they cover the largest area of the enclosure. It must also be ensured that the resolution of the camera images is sufficient enough to distinguish between individuals and that detail, such as the mouth, is clearly visible.
2. A second camera should be placed at least at a 90° (up to 180°) angle to the first camera to obtain video recordings from a different perspective, so that a possible obstructed view of one perspective can be resolved in the second perspective.
3. The audio stream should be gained from a microphone placed at a central location of the enclosure. The sampling frequency should meet the requirements of the ruminant species that is being investigated.
4. Special emphasis should be put into the synchronisation of video and audio signal as well as the synchronisation of the two videos. This could either be achieved by using specialized software that is able to delay the start of the video that started in delay compared to the other or manually. The manual setting requires more time and might cause several restarts. The synchronisation of the audio to the video happens normally during the recording, but – depending on the recording devices – it has to be done afterwards. A synchronized timestamp on the video and audio helps a lot with synchronisation.
5. Following the experimental phase, audio-visual material obtained should be analysed. The material can be analysed using a specially designed software or by counting behavioural/vocalisation events manually. Videos from at least two different angles should be analysed simultaneously. In most cases, it is useful to identify and record not only the vocal behaviour but the situational context (related behaviours) and physiological parameters.
6. The gained audio data should be examined regarding ambient noise, sound quality and overlapping vocalizations by group members. Afterwards the audio signal can be analysed using special software according to the designated scope.

B – Technical improvements

Analysing audio-visual recordings is a time-consuming task, so therefore some technical improvements may be necessary, especially in rarely vocalizing species like cattle. These technical improvements may need technical experienced staff and/or advanced programing skills.

1. Filtering the recordings for signals above a certain threshold can reduce the amount of irrelevant audio collected and therefore improve the speed of analysis. Event-oriented recording means that only events above a pre-set threshold are recorded, whereas times with noise levels below this threshold are excluded. To obtain recordings of whole vocalisations, which might begin below the

threshold, a buffer should be considered. The advantage of buffering is that a predefined time prior to when the threshold has been exceeded is also recorded. Buffering allows for whole vocalisations to be recorded but also can provide more context related behaviour. Limitations to this event oriented recording are noisy environments (noisy housing equipment, farm machinery, milking machines) and the probability that animals emit vocalisations below the pre-set threshold [19].

2. Gaining samples of vocalisations from each subject individually and analysing these vocalisations for their individual characteristics [20] could make future audio analysis quicker. From these individual characteristics, audio recordings obtained from the research site can be analysed and assigned to individual subjects without video analysis. However, gaining a sufficient number of audio samples per subject has some challenges. Firstly, there is a need to gain the audio samples in a controlled environment, as much as is possible. Ambient noise (i.e. birds, other herd members), sound wave reflections and noises from the subject's movements should be as low as possible and the distance to the recording unit should be as constant as possible. The second challenge is that collecting a vocalisation can be a time-consuming and demanding task, as some individuals will not produce a vocalisation, even during isolation in new surroundings. Therefore, it may be necessary to plan more than one recording session per animal.
3. Attaching microphones to the animal via their collar to obtain individual recordings can also reduce time spent analysing large data sets. For animals in herds, two microphones must be used; one with steady contact to the animal's neck to collect individual vocalisations and one which will collect ambient sound. Data from both microphones can be combined and an individual assignment can be made for any vocalisations. An advantage of this technique is that recording and assignment can be done without any prior steps. There are some disadvantages however, in that the data has to be collected for each animal individually, algorithms have to be programmed, and some elements of vocal communication can be cut off. At the moment this is an experimental approach but not a commercially available solution [21].

Pregnancy examination

Prerequisites

This guideline describes steps on performing pregnancy detection in cattle in an experimental unit. Steps on handling and recording pregnancy data associated with animal's pregnancy have been provided by the International Committee for Animal Recording (ICAR; https://www.icar.org/index.php/icar-recording-guidelines/). The present guideline assumes that the pregnancy test will be performed by either rectal palpation (35–42 d post insemination) or ultrasonography (28–32 d post insemination). The Animal Trait Ontology for Livestock (ATOL) numbers linked with this guideline are: **ATOL_0001032, ATOL_0001040,** and **ATOL_0001038** (for complete list of ATOL please visit https://www.atol-ontology.com/en/erter-2/).

A – General points

1. The animal's uterus should be examined (by either the rectal palpation or the ultrasound method) on 15–20 and 30–35 d post-calving to be sure that there are no signs of infection and the cow is ready for further reproductive processes.
2. Data associated with pregnancy in cattle such as calving to conception interval, conception (pregnancy) rate and days open should be documented by staff for optimal fertility management.
3. Animals must be properly restrained prior to the pregnancy check.
4. Handling and restraint should cause minimum stress to the animal.

B – Pregnancy test via recto-vaginal palpation

1. The palpating examiner should avoid harsh movements of the hand in the rectum, minimising any risk of injury to the animal.
2. Palpation should be avoided in animals with fever, as blood vessels are more fragile and bleed rapidly.
3. Examiners should be aware of sudden movements of the animal during examination in order to avoid injury, such as fractures of the arm.
4. Palpation of the uterine horns should be conducted carefully, using gentle pressure, to avoid rupturing the amniotic vesicle and terminating the pregnancy.

5. In case of being unable to detect any palpable pregnancy characteristics, the operator should re-examine the cattle 15–20 d later.
6. Signs of pregnancy which may be evident during palpation include an enlarged uterine horn containing placental fluid, foetal membranes, amniotic vesicles and the placentomes.
7. Examiners should have knowledge of bovine reproductive tract anatomy and have been trained on palpating the reproductive organs by experienced veterinarian or palpating operator.

C – Pregnancy detection using ultrasound examination

1. The ultrasound operator should have a good understanding of how to interpret ultrasound images, as well as being aware of possible artefacts that might result in misdiagnosis.
2. The operator must be aware during the ultrasound examination that fluid filled structures appear black (anechoic), hard structures (e.g. bone) appear white (hyperechoic) and other structures with their composition between the bone and fluid appears grey (hypoechoic). Diagnosis of pregnancy relies on ability to distinguish between these categories.
3. The operator should hold the entire transducer during examination with a constant and firm pressure, which is necessary for providing an optimal image.
4. The operator should not allow air into the rectum during the examination, as this will interrupt the sound waves.
5. A consistent routine of checking the uterine structure has to be developed by the examiner and followed for every pregnancy test of cattle.
6. If the ultrasound examination is carried out by a member of staff, they must have been trained by an experienced veterinarian/examiner on the use of ultrasound for pregnancy detection in cattle.
7. Equipment used must be washed and disinfected upon completion of examination.

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Citation note: Danesh Mesgaran S, Kuhla B, O'Donovan M, Schön PC, Röttgen V. Reproductive assessment. In: Mesgaran SD, Baumont R, Munksgaard L, Humphries D, Kennedy E, Dijkstra J, Dewhurst R, Ferguson H, Terré M, Kuhla B, (editors). Methods in cattle physiology and behaviour – Recommendations from the SmartCow consortium. Cologne: PUBLISSO; 2020-. DOI: 10.5680/mcpb003