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School reviewer: Professor of Veterinary Physiology

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Purpose

(1) The objective of this standard operating procedure is to provide guidance to the Charles Sturt University staff on:

a. Collection of faecal samples from live sheep

Scope

- (2) This procedure applies to any person who is involved in AEC approved projects involving collection of faecal samples from live sheep.
- (3) All researchers and teaching staff using animals for scientific purposes must be competent. For definition of competency refer to Charles Sturt University's Policy on 'Animal Care Competency Training and Assessment'

Details of procedure

Collection of faecal samples:

- (4) There are three main methods for collection of faecal samples from sheep:
 - i. Collection of samples from the ground after defecation (grab sampling)
 - ii. Collection of the samples directly from the rectum (per rectum)
 - iii. Collection of samples by bagging of the area around the anus

(5) Collection of samples from the ground after natural defection (grab sampling)

This is the least invasive method of sampling where faecal samples are collected after natural defecation from the ground or pasture on which the sheep were residing. Samples collected by this method can be kept as individual animal samples or pooled as a mob or flock level sample. For mob or flock level sampling, usually a minimum of 10 separate samples are collected, or approximately 10% of the flock whichever is greater, and pooled for sample analysis.

(6) Collection of samples per rectum

Collection of samples per rectum requires restraint of the animal during the procedure. In this instance, the sheep is confined in a race or crate, usually during a routine management procedure, and a sample of faecal material is collected directly from the rectum. Samples can be collected from some, or all the animals in a mob, or from selected animals according to the requirements of the AEC approved protocol.

The procedure is undertaken as quickly as possible, using a gloved, lubricated hand with one or 2 fingers inserted into the rectum up to 5-7cm, and a sample of faecal material removed. The ease of access can be increased by application of lubricant jelly prior to insertion although this may only be required under drought feeding conditions. Samples should be



collected as soon after yarding as possible and usually within 4 hours. If no sample is immediately palpable on insertion, the animal can be left for 1-2 minutes and a further exploratory investigation attempted, frequently the stimulation of the anus results in defecation and therefore often samples can be present on the second inspection. If no sample is present on a second inspection, then the rectum should be considered empty, and the animal recorded as not having produced a specimen. If samples are to be collected from multiple animals, then either new gloves should be used for each animal, or gloved hands cleaned in soapy water between animals and gloves changed within cohorts. See the 'Paraboss' sampling protocol for more information.

(7) Collection of samples using faecal bags

This collection method should only be used where sheep are in a high-containment facility with close monitoring, or in a highly managed extensive setting where daily observation and yarding is possible.

Where sample collection may need to occur to determine total faecal volume over time, or for extended non-invasive monitoring, faecal bags can be used. In this case, a plastic bag (Ziplock or similar) can be attached to the fleece at the rear of the animal using adhesive (glue or tape, although superglue is most effective) with the opening of the bag located across under the tail (in long tailed animals) or above the tail (in docked animals) and anal opening. This can be done either by an operator entering the confinement crate with the animal, securing the sheep against a fixed partition with their upper leg and bodyweight, then reaching over the back of the animal to secure the bag, or using a sheep crush or race to contain the animal whilst the bag is being applied. As soon as the bag is applied the operator can release the animal into the field or pen.

Faeces will be collected by forces of gravity into the bag at defection. At the end of the collection period the bag is carefully removed by cutting the fleece to which it is adhered.

Bags are usually retained on the animal for no more than 24 hours and should be removed after use.

Drugs, chemicals, or biological agents

(8) Not applicable.

Impact of procedure on wellbeing of animals

- (9) For grab sampling, the impact on the animals is negligible. This technique requires minimum disturbance of sheep from their natural behaviours, and only may impact them due to an unfamiliar (or likely familiar) person or vehicle in the paddock or pen for a short period of time.
- (10) For per rectum sampling, this is a minimally invasive procedure and requires restraint of the animal for a short period of time usually less than 1 minute. Discomfort with the procedure can be minimised by ensuring smooth ease of access to the rectum, with a lubricated glove, and low stress handling techniques prior to and after the procedure.



(11) Faecal bag sampling is also a minimally invasive technique, and from a behavioural view less invasive that per rectum sampling. The period of discomfort will only be during application and removal of the bag, neither of which requires an invasive procedure. Faecal bags can be either purpose designed sheep collection bags for extensive in-paddock monitoring (<7 days), or plastic bags secured to the rear of the animal for short term monitoring (<24 hours). Although application of faecal bags can take several minutes to ensure that the adhesive has secured the bag in position, removal can be rapid taking only a few seconds for an experienced operator. Again, the use of low stress handling techniques prior to and after removal will minimise impacts on the animal.

Animal care

- (12) Low stress management procedures should be applied in all three cases, with minimal handling of sheep during any of the procedures. Grab bag sampling requires no handling, and handling in procedures for per rectum and faecal bag applications can be kept to a minimum.
- (13) For per rectum sampling, if repeated samples are to be taken it is critical that lubrication is used to minimise the likelihood of damage to the anus or rectal issues.
- (14) Observation of any animal with a faecal bag attached should occur at regular intervals (every 6-8 hours, except overnight where additional observations could disturb normal sleep behaviours) to ensure that the bag has remained attached. Bags that have become naturally attached should be collected from the area as soon as possible to as not to cause an ingestion hazard (although due to the nature of the contents this is unlikely).

Pain relief

(15) Pain relief is not required for any of these procedures.

Reuse and repeated use

- (16) Reuse and repeated use of sheep for faecal monitoring, usually by grab sampling, is a required part of good sheep management as this technique is routinely used for parasite burden analysis and anthelminthic treatment management. It is likely that most, or all sheep will have experienced this procedure during their lifetime.
- (17) Animals involved in trials requiring per rectum sampling may also undergo this minimally invasive technique several times. If good handling practice is used, the technique can be applied many times a day, every day, or with any frequency interval without significant welfare impact. Sheep undergoing per rectum sampling are likely to have also undergone grab sampling at some point in their management history. The two are not likely to be cumulatively impactful or exert a negative welfare outcome if good handling and competent operators are involved.
- (18) Animals involved in trials requiring faecal bag sampling are unlikely to experience this on multiple occasions. If good handling practice is used, the technique is minimally invasive but is usually restricted to a specific time-period within a project or trial. It is possible that this is repeated on multiple occasions for the same animal, but this would be approved under a AEC protocol that identifies how repeated exposures would be managed for minimal impact



on animal welfare. In this case, the context of the housing, other experimental conditions that might be applied, and the repeated exposure to humans and handling events are likely to be the most impactful measures, not the faecal bag procedure itself. Sheep undergoing faecal bag sampling may also have undergone par rectum sampling and are likely to have also undergone grab sampling at some point in their management history. These procedures are not cumulatively impactful or exert a negative welfare outcome if good handling and competent operators are involved in their execution and delivery.

Qualifications, experience or training necessary to perform this procedure

- (19) Grab bag sampling can be undertaken by persons trained in good animal management but without the requirement for experience in animal handling.
- (20) Per rectum sampling should only be undertaken by a competent person, or person in training under the supervision of a competent operator.

Record requirements

(21) Where an individual animal is known, a record of either per rectum or faecal bag sampling should be recorded against that animal's trial records and according to AEC reporting requirements.

Associated documentation (including pictures if available)

(22) Not applicable

Glossary

(23) Not applicable

References and relevant links

(24) Collecting dung samples from sheep and goats – Paraboss procedure.