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RESEARCH NOTE

PRELIMINARY TRIAL ON THE USE OF BARIUM CHLORIDE FOR PREGNANCY DIAGNOSIS IN GOATS

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ABSTRACT

The absence of farm records limits the application of artificial insemination (AI), and risk in hormone administration to synchronize estrus is very high. Although there are available means to detect pregnancy, availability and cost of equipment prevent utilization. Hence, the possible application of a non-invasive technique in pregnancy detection among goats was evaluated. A total of 567 does were subjected to pregnancy diagnosis using urine as test sample and 5% barium chloride ($BaCl_2$) as test solution. Urine samples were collected in the morning using collection bags. Samples with precipitation indicate pregnancy; while those that remain clear show that the doe is dry. The result of the diagnosis using Confusion Matrix shows that $BaCl_2$ is 62% accurate in classifying pregnant and non-pregnant does. Moreover, its specificity is 49% and precision is 48%. This study confirms that $BaCl_2$ can be used for non-invasive pregnancy diagnosis in goat.

Key words: pregnancy diagnosis, non-invasive technique, barium chloride, goat

INTRODUCTION

The use of artificial insemination (AI) among goats has been promoted as an alternative option in upgrading particularly in backyard farms. However, the absence of farm records limits its application. As a protocol, early diagnosis of pregnancy is required to identify which doe is to be synchronized and avoid the risk of abortion. Moreover, if AI is executed, pregnancy detection is needed to determine which among the does inseminated did not successfully conceive and thus needed to be inseminated again to reduce production time lost (Lalrinthluanga and Dutta, 2009).

At present, the use of the ultrasound method to diagnose pregnancy is becoming popular among livestock raisers (Omontese *et al*., 2012). A-mode ultrasonography is based on ultrasound wave reflection that determines the boundaries of an organ filled with liquid; Doppler ultrasonography investigates blood circulation in median uterine artery, umbilical artery and fetal heartbeat as studied by Serin *et al*. (2010) and B-mode real-time

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ultrasonography can generate two-dimensional image display composed of bright dots representing the ultrasound echoes (Erdogan, 2012). To establish the functionality of these techniques particularly in goats, several studies have been conducted. For instance, in the study conducted by Dawson (2002) using A-mode ultrasonography, good sensitivity and specificity can be obtained between 60 to 120 days after breeding, with at least 85% accuracy. On the other hand, the accuracy of Doppler ultrasonography via transrectal or transcutaneous approach is higher compared with the result obtained using A-mode ultrasonography at 60 to 90 days after breeding (Serin *et al*., 2010). Meanwhile, in the study conducted by Amer (2010), it was found that screening of embryonic vesicles can be detected at 21 to 28 days and the embryo can be visualized at 28-35 gestation days using transabdominal uterine scans. However, since these techniques are costly and need a skilled person to work with, these methods are not being used by the majority of the raisers.

Aside from generating images, pregnancy in goats can also be determined through progesterone-based assays using blood, milk and fecal samples (Williams, 1986; Wani *et al*., 2003). These methods require laboratory equipment for enzyme-linked immunosorbent assay (ELISA), radioimmune assay (RIA), or immunocheminescence analysis (Karadaev, 2015). In the study conducted by Boscos *et al*. (2003), blood serum analysis can be used as an indicative measurement of pregnancy after 21 days of insemination. Moreover, using milk progesterone, Dawson (1999) found that when concentration is greater than or equal to 10ng/ml, pregnancy is indicative and can be detected 22-26 days after breeding. The experiment of Jack *et al*. (2002) shows that measuring progesterone in does to conclude pregnancy is 100% accurate when done 19-20 days after mating. Similarly, these techniques have high accuracy but requires specialized laboratory equipment and testing is costly.

A cheaper method of detecting pregnancy is through the use of barium chloride ($BaCl_2$) solution. This was shown to work in detecting pregnancy in ewe (Ohazurike, 1990), pigs (Ndu *et al*., 2000) and cattle (Elpakov and Tsyganok, 1966). The use of non-invasive pregnancy detection is carried out by the presence of progesterone in the body fluid of the animal. Physiologically, progesterone is secreted continuously to maintain the developing embryo. Progesterone will react with $BaCl_2$, as shown by the presence of precipitate (Holness, 1991 as cited by Lalrintluanga and Dutta, 2009). $BaCl_2$ is an inorganic compound and one of the most common water-soluble salts of barium. When urine is added to $BaCl_2$, it combines with sulphate radicals in urine and precipitate of barium sulphate is formed. This method will not require highly technical skills, as compared when visual image or acoustic is used. Furthermore, this method is can be applied on field-level, thus the application of synchronization to doe subjected to artificial insemination can be more efficient. This study was therefore conducted to screen the applicability of non-invasive urine-based pregnancy diagnosis in goats using $BaCl_2$.

MATERIALS AND METHODS

A total of 567 does from different backyard farms in Echague, Isabela was used as sample donors in this study. The urine samples were collected in the morning using a urine collecting bag. The collected sample was added to 5% barium chloride solution using the ratio 1:1 (sample: test solution). The presence of precipitates in the mixture indicates that the doe is pregnant; otherwise, the doe is dry.

The principles of a confusion matrix table were adopted to describe the performance of the classifier on a set of test data. The matrix is usually used in studying the performance of developed medical kits by evaluating the accuracy of a test classifier (Kandhasamy and Balamurali, 2015).

For this particular study, the classifier is the test solution which is barium chloride. To evaluate the accuracy of the result of the pregnancy diagnosis using the following data classification:

True positive (TP)	these are cases in which the doe is known to be pregnant, and pregnancy is confirmed true during the pregnancy diagnosis using the test solution.
True negative (TN)	these are cases in which the doe is known to be dry (non-pregnant) and the status was confirmed using the test.
False positive (FP)	these are cases in which it was known that the doe was dry, but the test result confirms that the doe is pregnant
False negative (FN)	these are cases in which it was known that the doe is pregnant, but the test result confirmed that the doe was not pregnant or dry.

Using the Confusion Matrix, the following rates were computed to determine the accuracy of the test classifier:

Accuracy is the number of all correct prediction over the total number of population tested, computed as TN+TP/n.
Error rate is the number of incorrect predictions over the total data set, computed as FP+FN/ total .
Specificity is the number of correct negative predictions, computed as TN/actual no.
Precision is the number of correct positive prediction, computed as TP/(TP + FP).

Non- return of heat was monitored and observed in support of the experiment conducted on non-invasive pregnancy testing. For the observation of heat, the animals that tested positive when subjected to non-invasive pregnancy analysis were monitored for the return of heat after 21 days.

RESULTS AND DISCUSSION

Using 5% barium chloride ($BaCl_2$), the result revealed that from 567 samples, 171 were regarded as true positive while 180 were classified as true negative. There were 185 false positive data and 31 were regarded as false negative.

Using the Confusion Matrix Table (Table 1), it shows that 5% barium chloride as test classifier has 62% accuracy in classifying pregnant and non-pregnant does at an error rate of 38%. Moreover, the specificity rate is computed at 49%; while the precision rate is 48%. From this data classification set, it shows that 5% $BaCl_2$ can be used in classifying pregnant and dry doe using urine at 62% accuracy. This result is higher than the data reported by Ndu *et al*. (2000) in detecting pregnant sows at 59% accuracy. The results also confirm that $BaCl_2$ can detect for the presence of progesterone progesterone among pregnant using urine as a sample (Lalrintluanga and Dutta, 2009).

Table 1. Confusion Matrix for pregnancy detection using barium chloride.

	Predicted: NO	**Predicted: YES**	**Total**
Actual: NO	(TN) 180	(FP) 185	365
Actual: YES	(FN) 31	(TP) 171	202
Total	211	356	567

TN - true negative, FN - false negative, FP - false positive, TP - true positive

As also revealed in the study, there are 185 does that are being identified by raisers as pregnant; however, the test result revealed that the does are dry. This incorrect perception of the status of the animal will add to the low productivity of the doe as a chance to reproduce is missed. On the other hand, the test result also shows that there are pregnant does in which the raisers thought to be dry. Without proper knowledge on the status of the animal, improper production management may be implemented, which may result in abortion, low milk production, weak kids and even mortality. Hence, the mechanism for pregnancy detection is necessary to improve animal as well as farm production performance.

Therefore, the present study shows that 5% barium chloride solution can be applicable in conducting non-invasive pregnancy screening among goats. Considering the cost, laboratory and technical skill requirements of the available technologies like ultrasonography and progesterone-based assay, utilization of low-cost, easy to use and applicable at field-setting like the use of non-invasive technique can be further explored in lieu to the existing techniques.

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