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Comparative diagnosis of pregnancy wastage in cows at slaughter using pregnancy specific protein-B and post slaughter inspection diagnostic procedures

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ABSTRACT

Slaughter of pregnant animals is a common abattoir practice. This study was designed to compare the diagnostic capacity of pregnancy specific protein-B (PSPB) with post slaughter inspection (PSI) procedures in detecting cows pregnant at slaughter. Blood was collected from cows presenteded for slaughter at an abattoir. The uteri were examined post slaughter for the presence or absence of a foetus. Recovered foetuses were aged using crown-rump length to estimate the stage of pregnancy. Of the 361 cows examined, 72 (19.9 %) were diagnosed pregnant using the PSPB procedure, while 32 (8.9 %) were diagnosed pregnant using PSI diagnosis. Furthermore, with PSI there was a lack of pregnancy diagnosis in 42 (11.6 %) cows detected pregnant using PSPB procedure, and two (0.6 %) cows detected pregnant using PSI were not detected to be pregnant using PSPB diagnosis. Validity of the diagnostic procedures indicated that sensitivity and specificity of the PSPB was 93.8 % and 87.2 %, respectively, while with the PSI diagnosis there was a sensitivity and specificity of 41.7 % and 99.3 %, respectively. The PSPB diagnosis, had an excellent predictive value (AUC – 0.92; $p < 0.001$; 95 % CI – 0.856 to 0.981). Most of the pregnancy wastage ($n = 22$; 68.8 %) diagnosed using the PSI method were in the second trimester. The results of this study indicate that PSPB is reliable and a more sensitive diagnostic method than PSI . It is therefore recommended that the PSPB test be incorporated in routine screening for pregnancy status of cows before slaughter.

1. Introduction

There are cattle throughout Nigeria but most common in the northern two-thirds of the country, where cattle are important sources of protein; providing meat and milk (Blench, 1999). In addition, the hide and skin of cattle are beneficial, while the blood and horns are used for animal feed (Bourn, 2010). Cattle are also used for transportation and in agriculture for ploughing, harrowing, ridging and lifting of water from deep wells (Blench, 1999). Cows have an important role in cattle production as reprpductive vessels by carrying pregnancy (Umaru et al., 2009). Pregnancy wastage due to slaughter of pregnant animals, however, is a regular occurrence in the abattoirs of many developing countries such as Nigeria. This is a hindrance to sustainable cattle production due to wastage of animals that would produce valuable resources (*e.g.*, meat and milk) had the cows not been slaughtered.

In Nigeria, prevalence of pregnancy wastage in cattle has been reported to range from 1.5%–10.3% (Ibironke, 2010; Alhaji, 2011;

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Bokko, 2011; Ngbede et al., 2012; Akpabio and Babalola, 2014; Odeh et al., 2015; Hassan et al., 2016; Ogunbodede and Oladele, 2016). It has also been reported in sheep and goats (Addass et al., 2010; Bokko, 2011; Adeyeye et al., 2019), camels (Bello et al., 2008) and pigs (Amuta et al., 2018). Pregnancy wastage as a result of slaughtering pregnant animals has also been reported in other African countries such as Ghana (Atawalna et al., 2013), Ethiopia (Simenew et al., 2011) and Zambia (Zulu et al., 2013).

Diagnosis of pregnancy wastage in most of these studies was based on post slaughter examination involving palpation, incision and visual inspection. The uterus of the cow is removed after slaughter, palpated for a foetus, incised along the uterine horn and visually inspected for the presence or absence of foetuses. Another method used for diagnosis of pregnancy wastage is the flushing of the uterus immediately after slaughter. This method comprises retrograde flushing of embryos from the uterus immediately after slaughter to recover pre-implanted or implanted embryos (Hamman et al., 1997).

The PSPB compound also known as Pregnancy Associated Glycoprotein (PAG) or Pregnancy Serum Protein 60 (PSP-60) (Sousa et al., 2008), initially described as placental antigens are present in maternal circulation after implantation (Sousa et al., 2006). The PSPB are proteins synthesized in the mono- and bi-nucleate cells of the ruminant trophectoderm (Sousa et al., 2006), from where these proteins are released into circulation and their presence used in monitoring pregnancy status (Karen et al., 2003) and some obstetric diseases (Adeyeye et al., 2016). These proteins are important to clinicians and researchers studying the pathophysiology of pregnancy in ruminants (Breukelman et al., 2005). Apart from Adeyeye et al. (2019) where there was comparison of PSPB diagnosis with post slaughter inspection in sheep, no study has been conducted, to the best of our knowledge, in assessment of pregnancy wastage as a result of animal slaughter. The present study was designed to compare the diagnostic capacity of PSPB with post slaughter inspection in detecting pregnant cows at slaughter. Information obtained from this study will help farmers adopt a reliable method for pregnancy diagnosis in cows presented for slaughter, thereby preventing pregnancy wastage and enhancing the productivity of the farmer and cattle industry.

2. Materials and methods

2.1. Study location

The study was conducted at the Sokoto Modern Abattoir and the facilities of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria. Sokoto state is located between 13°and 14 °N and between 5° and 6 °E. Cattle, sheep, goats and camels are slaughtered at the Sokoto Modern Abattoir with an average daily slaughter of 158 cattle (82 cows and 76 bulls). Laboratory processing and analysis of blood samples were conducted at the Central Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.

2.2. Animals and ethical approval

The cows used for this study are indigenous breeds brought for slaughter at the Sokoto Modern Abattoir. The majority of these animals are purchased from different parts of Sokoto State, while others are from neighbouring Niger Republic, which is about 250 km (about 156 miles) from Sokoto metropolis.

Approval for this study was obtained from the Sokoto State Ministry of Animal Health and Fisheries Development, the supervisory ministry for the abattoir. During each diagnostic session at the abattoir, verbal consent was obtained from the owner of each cow prior to sampling.

2.3. Study design

A total of 361 cows slaughtered at the Sokoto Modern Abattoir between September and November 2018 were selected using a convenient sampling method. About 5 ml of blood were collected at slaughter in a non-heparinized tube, labelled appropriately and transported on ice pack to the laboratory for analysis. Uteri of sampled cows were palpated, incised and visually inspected after slaughter for the presence or absence of foetus.

2.4. Age of pregnancy wastage evaluations

The age of pregnancy wastage was determined by estimating the gestational age of the foetus using the crown-rump length as described by Richardson (1980). Using an average gestation length of 283 days, the foetal development stage was classified into trimesters as first (1–93 days), second (94–188 days) and third (189–283 days).

2.5. ELISA assay for PSPB

The blood collected from each cow was centrifuged at 1677*g* for 5 min and the serum was collected. The PSPB was assayed using BioPRYN® ELISA test kits manufactured by BioTracking LLC, Moscow, ID, USA. It is a qualitative ELISA kit used for detection of PSPB in sera or plasma of pregnant cows, ewes, goat does and buffalo cows. The assay was conducted as described by the manufacturer. Briefly, all reagents were mixed thoroughly, warmed with the plates at room temperature and 50 µl of the detector solution was added to each well of the PSPB antibody coated plate. About 150 µl of serum samples were loaded to an uncoated transfer plate, of which 100 µl were transferred simultaneously to the coated plates using a multi-channel pipette. The same 100 µl of the standards

(Ho and Lo) were also loaded into the coated plate, sealed and incubated for 1–2 h at room temperature. After the incubation, the contents of the plates were discarded and washed four times using 200 µl of distilled water and blot dried each time. About 100 µl of enhancer solution was added to each well and incubated for 30 min. The plate was washed again with 200 µl of distilled water and blot dried each time as described above. About 100 µl of 3,3′,5,5′-Tetramethylbenzidine was added to each well, sealed, mixed gently and incubated for 15 min at room temperature. Stop solution was added to the plate and readings occurred within 30 min on an ELISA plate reader to determine the optical density (OD) of the test at a wavelength of 630 nm. Results obtained were categorized as positive or negative based on the value of the OD, compared to the standard ODs (high - Hi and low – Lo). Sample OD less than "Lo" were classified as "not pregnant", while samples with ODs greater than "Hi" were classified `pregnant`. Those with OD values between "Lo" and "Hi" were re-analysed.

2.6. Validity of diagnostic test

The validity of the test was determined by calculating the sensitivity and specificity of each diagnostic test. Sensitivity indicates the accuracy of the diagnostic test in detecting pregnant cows while; the specificity indicates the accuracy of the test in detecting non-pregnant cows. This was calculated using the following:

a True positive: cow was pregnant at slaughter and tested positive for PSPB test;
b False positive: cow had no foetus at slaughter but tested positive for PSPB test;
c True negative: cow had no foetus at slaughter and was negative for PSPB test; or
d False negative: cow had foetus at slaughter but tested negative for PSPB test.

The sensitivity was, therefore, expressed as, $= \frac{a}{a+d} \times 100$, while the specificity was expressed as: $\frac{c}{c+b} \times 100$.

2.7. Data analyses

Data obtained were used to calculate the validity of the diagnostic test. Kappa statistics were used to determine the consistency between PSPB and post slaughter inspection at a significant level of 5 % and confidence interval of 95 %. In addition, a Receiver Operating Curve (ROC) was used to validate the PSPB test. The stage of pregnancy of recovered foetuses were subjected to descriptive statistics.

3. Results

The data for comparative analysis using PSPB and post slaughter inspection procedure are presented in Table 1. A total of 72 (19.9 %) cows were diagnosed pregnant using PSPB procedure, while 32 (8.9 %) cows were diagnosed pregnant using post slaughter inspection procedure. With post slaughter inspection procedure for pregnancy diagnosis, there was a failure to diagnose 42 (11.6 %) cows diagnosed pregnant using PSPB procedure, while 2 (0.6 %) cows detected pregnant using post slaughter inspection procedure were undetected using PSPB procedure. The data for validity of the two diagnostic tests in diagnosing pregnancy at slaughter are presented in Table 2. The sensitivity and specificity of the PSPB test was 93.8 % and 87.2 %, respectively, while post slaughter inspection had a sensitivity and specificity of 41.7 % and 99.3 %, respectively. The measure of agreement was moderate (K = 0.52).

The ROC curve is presented in Fig. 1. The curve showed an excellent predictive value (AUC – 0.92; $P < 0.001$; 95 % CI – 0.856 to 0.981) and a cut off value of 0.3050 (sensitivity – 90.6 %; specificity – 90.3 %) for PSPB diagnostic test. Based on post slaughter inspection, the data for stage of pregnancy of cows slaughtered are presented in Table 3. A total of 22 pregnancies were detected that were at the second trimester stage of pregnancy while, five each were detected that were in the first and third trimester stages of pregnancy.

4. Discussion

A greater rate of pregnancy wastage was diagnosed using PSPB than post slaughter inspection. This is similar to the results reported by Adeyeye et al. (2019) in ewes at slaughter. The PSPB diagnostic test can be used to detect pregnant cows as early as day 28 of gestation, unlike the post slaughter method which can be used to only diagnose pregnancy after organogenesis. Post slaugter

Table 1
Comparative analysis of pregnancy specific using Protein-B and post slaughter inspection diagnostic procedures in detecting pregnancy wastage in cows at slaughter.

		Post slaughter inspection		Total
		No	Yes	
PSPB	No	287 (79.5%)	2 (0.6%)	289 (80.1%)
	Yes	42 (11.6%)	30 (8.3%)	72 (19.9%)
Total		329 (91.1%)	32 (8.9%)	361

Table 2
Sensitivity and specificity of pregnancy specific Protein-B and post slaughter inspection procedures in diagnosis pregnancy wastage of slaughter cows (n = 361).

	PSPB	Post Slaughter
Sensitivity	93.8%	41.7%
Specificity	87.2%	99.3%
Kappa Value	0.52	

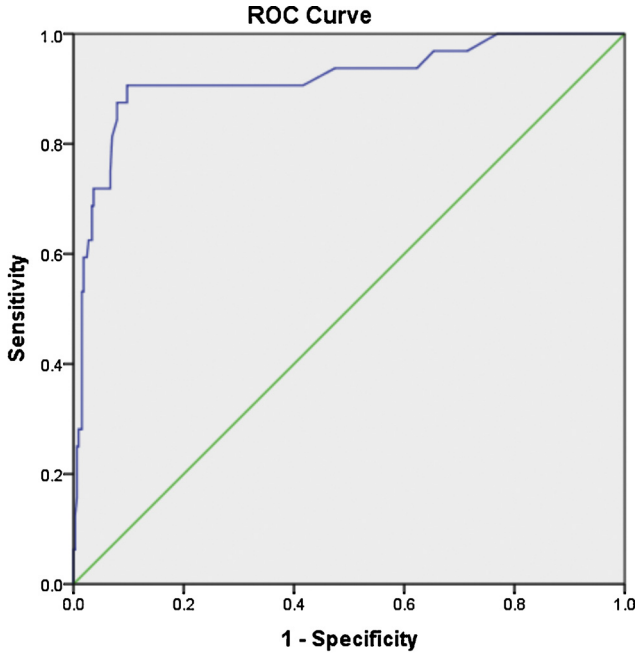

Fig. 1. Receiver operating curve (ROC) of pregnancy specific Protein-B in detecting pregnant cows slaughtered at Sokoto Modern Abattoir.
AUC – 0.92; p > 0.001; 95 % CI – 0.856 to 0.981.
Cut off value – 0.3050 (sensitivity: 90.6 %; specificity: 90.3 %).

Table 3
Stage of pregnancy of cows slaughtered at Sokoto Modern Abattoir using post slaughter inspection procedures (n = 32).

Stages of Pregnancy	Number
First trimester	5
Second trimester	22
Third trimester	5

method involves palpation and incision of the female genitalia after slaughter, followed by visual inspection for the presence or absence of foetuses. Post slaughter inspection only provides for post slaughter examination in which the animal is dead, unlike PSPB suggesting that more pregnant cows may have been slaughtered compared to what was reported in earlier studies that relied solely on post slaughter inspection for pregnancy diagnosis (Akpabio and Babalola, 2014; Odeh et al., 2015; Hassan et al., 2016; Ogunbodede and Oladele, 2016).

The measure of consistency between PSPB and post slaughter inspection determination in diagnosing pregnant cows at slaughter was moderate implying there is not a great amount of consistency when the two methods for pregnancy diagnosis are used. There was a similar observation in ewes by Adeyeye et al. (2019). This lack of consistency of pregnancy diagnosis when the two methods is used may probably be due to the long half-life of PSPB in blood that makes it detectable long after embryonic or foetal death in cattle (Zoli et al., 1992). In the present study, however, the PSPB test was more sensitive in diagnosing pregnancy than post slaughter inspection procedure, although post slaughter inspection was more specific than PSPB. The lack of sensitivity of the post slaughter inspection

method compared to PSPB procedure may indicate that this method is less accurate in diagnosing pregnant cows, which is a limitation. The AUC value that was determined in the present study indicates that PSPB has an excellent predictive value in predicting pregnant cows at slaughter, and as such is a reliable screening test.

Post slaughter inspection revealed that the greatest rates of pregnancy wastage occurred in the second trimester compared to the first and third trimesters of pregnancy. These findings are consistent with those from earlier studies on stage of gestation in pregnancy wastage of cows (Ogunbodede and Oladele, 2016; Raimi et al., 2017). In the present study, however, there were 42 cows detected pregnant using PSPB procedure that were detected not pregnant using post slaughter inspection procedure, probably because these cows were in the embryonic developmental stage of pregnancy when organogenesis had not yet commenced. These cows may, therefore, be classified as having pregnancy wastage indicating there was greater pregnancy wastage due to slaughter of pregnant cows in the first trimester than during other trimesters, and further indicates the capacity of PSPB procedure to detect early pregnancy. The studies of Ngbede et al. (2012); Odeh et al. (2015) and Alhaji et al. (2017) used post slaughter inspection procedure alone and reported more pregnancy wastage in the first trimester. This may mean that these first trimester pregnancy wastages were under reported in previous studies, because of reliance on visual assessment of pregnancy which may not be apparent during the early stages of pregnancy and not detected to be pregnant at the time of slaughter.

5. Conclusion

In conclusion, the results from the present study indicate that PSPB procedure is more sensitive in diagnosing pregnancy of cows at slaughter than post slaughter inspection procedure. In addition, the PSPB procedure is a reliable diagnostic test for detecting pregnant cows at slaughter. This procedure, therefore, is a recommended test to be included in routine ante mortem screening of cows for pregnancy at slaughter. This will aid in diagnosing early pregnancy and subsequent isolation of pregnant cows. It will also minimize the error associated with post slaughter inspection of pregnancy and reduce the risk of pregnancy loss. Furthermore, use of PSPB test in routine pregnancy diagnosis on farms and at abattoirs will aid in more accurate detection of pregnancy, in cows, particularly in early stages thereby increasing productivity of the livestock industry.

Declaration of Competing Interest

None.

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