Global Veterinaria 21 (2): 48-52, 2019
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2019.48.52

Comparative Detection of Pregnancy in Ewes at Slaughter Using Pregnancy Specific Protein-B and Post Slaughter Examination

Adewale A. Adeyeye, Musa I. Abubakar and Ahmed G. Ahmed

Department of Theriogenology and Animal Production,
Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria

Abstract: This study was designed to compare assay for pregnancy specific protein-B (PSPB) and post slaughter examination for detecting pregnancy in ewe. From 67 ewes presented for slaughter at a slaughter house in Sokoto, Nigeria, 5 mL of blood were collected to assay PSPB, then after slaughter, their uteri were examined for the presence or absence of foetuses. The sex of all recovered foetuses was determined and the crown-rump length was recorded to estimate the age. The pregnancy status revealed that out of 67 ewes sampled, 27 (40.3%) were detected pregnant by PSPB assay, while only 10 (14.9%) were pregnant by post slaughter examination. The comparison between the two diagnostic tests showed that PSPB was 100 % sensitive while, post slaughter was 37 % sensitive. The specificity was 100 % and 85.1 % for PSPB and post slaughter examination, respectively. PSPB was 100 % accurate, while post slaughter was 74.6 % accurate in detecting pregnant ewes at slaughter. Their kappa value was 0.41 suggesting a moderate agreement. Based on post slaughter examination. 11 (10 singleton and 1 twin) foetuses made up of 7 (63.6%) females and 4 (36.4%) males were recovered. Majority of them were in second trimester, 8 (72.7%) than first trimester 2 (18.2%) or third trimester 1 (9.1%). Categorization of test results from PSPB assay showed that apart from the 27 (40.3%) that were pregnant, 31 (46.3%) were not pregnant, while 9 (18.2%) needed to be rechecked. However, this was not possible since they were not available. The study shows that pregnancy specific protein-B detected pregnancy better than post slaughter examination.

Key words: Ewe · Post slaughter examination · Pregnancy · Pregnancy Specific Protein-B · Sokoto

INTRODUCTION

Sheep is a ruminant animal principally kept for meat and milk in Nigeria [1]. In the northern part of the country, they are important for religious and socio-economic purpose, where they are reared and used during festive periods such as *eld-el kabir*, weddings and naming ceremonies [2]. Nevertheless, this important role can only be sustained through reproduction and pregnancy in an integral part. Pregnancy is the interval from fertile mating to parturition and it begins immediately after fertilization [3]. In the sheep, the duration is 147-152 days and involves a multifaceted process including cleavage of the zygote, embryo formation and foetal development [4]. From literature, the duration is influenced by maternal, foetal, genetic and environmental factors [3]. However, embryonic death or foetal loss from abortion or stillbirths can adversely influence pregnancy [5]. In addition, the continuous slaughter of pregnant female animals particularly in developing countries shortens the duration of pregnancy, thereby reducing the animal protein available for human consumption as well as income of the farmer [6, 7].

Slaughter of pregnant animals is a common practice in Nigeria and other parts of sub-Saharan Africa, where prevalence rates depend on specie and location [8]. In Nigeria, the practice is not only prevalent for sheep and goats [8 - 12] but also other animals [6, 13 - 17]. Pregnancy wastage through slaughter of pregnant animals has also been reported in other countries, with significant prevalence [7, 18 - 21]. All these wastage in pregnancy were determined using post slaughter examination of animals. This involves visual inspection of the uteri for the presence or absence of foetuses after slaughter.

Corresponding Author: Adewale A. Adeyeye, Department of Theriogenology and Animal Production, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.
Tel: +2348032859940.

The procedure is flawed due to its inability to detect early pregnancies where organogenesis has not occurred. Apart from this, when foetuses are detected, slaughter of the pregnant animal cannot be deferred till parturition. This study was therefore designed to determine the pregnancy status of ewes presented for slaughter at a house in Sokoto, Nigeria using pregnancy specific protein-B (PSPB) and to establish its superiority or otherwise over post slaughter examination as a better tool for assessing pregnancy status of ewes. PSPB constitute a large family of placental glycoproteins belonging to a group of proteolytic enzymes [22]. They are secreted by the superficial layer of the developing trophoblast of ruminants and released into maternal circulation [23]. Their concentrations are detected and used to determine pregnancy and obstetric diseases [24, 25]. Determination of PSPB levels in ewes prior to slaughter will significantly reduce pregnancy wastage and its associated financial loss.

MATERIALS AND METHODS

Study Location: The study was carried out at the Bassa-shuni slaughter slab, located 12.5 km (7.8 miles) from Sokoto the capital of Sokoto state, Northwestern Nigeria. It is one of the main slaughter houses for small ruminants in Sokoto state. An average of 30 animals is slaughtered at the slab daily, comprising 20 sheep and 10 goats. Blood samples were processed and analyzed at the Central Laboratory of the City campus, Usmanu Danfodiyo University, Sokoto, Nigeria.

Study Design: This was a prospective study of 67 ewes slaughtered at the Bassa-shuni slaughter slab from November to December, 2018. Permission for the study was obtained from the leadership of the animal sellers and butchers association, who are responsible for the sale and slaughter of animals at the slaughter slab. During each visit prior to sampling, consent of the owner of each ewe was obtained. Facilities for live weight recording were absent. However, their live weights were estimated using the methods described by Sowande and Sobola [26], while their breeds were determined based on the coat colour [27] and noted in a record book. About 5 mL of blood was collected at slaughter into plain sample bottles in order to assay for pregnancy specific protein-B. After evisceration, the uteri of the ewes were examined for the presence or absence of foetuses. The inguinal area was observed to determine its sex, while the crown-rump length was measure using a measuring tape and used to estimate the age of the foetuses as previously described [28].

Blood Collection and Handling: The blood collected was transported to the Laboratory on ice packs. At the Laboratory, the blood was centrifuged at 9, 000 g for 5 minutes using a Digital centrifuge (Model – Biofuge, Made in USA), serum was harvested and stored at -4°C until use.

ELISA for Pregnancy Specific Protein-B: The ELISA used is the bioPRYN flex produced by Bio Tracking Inc. It is an antigen-capture, or "sandwich, " Enzyme-Linked ImmunoSorbent Assay (ELISA) that detects Pregnancy Specific Protein-B (PSPB) in bovine, ovine and caprine serum/plasma. The protocol outlined by the manufacturer was followed. Briefly, 50 µL of detector solution was added to each well of the antibody coated plate. About 200 µL of sample serum were pipetted into a transfer plate, while 100 µL of this was transferred into the PSPB antibody coated micro-plates using a multi-channel pipette. In the same vein, 100 µL of PSPB standard Hi and PSPB standard Lo were added to appropriate wells, covered with foil paper and incubated for 2 hours at 18 – 24°C. After incubation, the plate was swirled for 5 sec, then foil was removed and the wells were washed 4 times using 200 µL of wash solution, dumping and blotting in each case. Using a multi-channel pipette, 100 µL of enhancer solution was added to each well, sealed and incubated for 30 minutes at 18 – 24°C. After this period, 200 µL of wash solution was used to wash each well 4 times as earlier described. TMB (100 µL) was added to each well and incubated for 15 minutes at 18–24°C. The reaction was stopped by adding 50 µL of stop solution using a multi-channel ELISA. Within 30 minutes of adding the stop solution, the plate was read using a plate reader of wavelength 630 nm. Results were categorized based on the optical density (OD) of the sample and compared with the mean of the Hi and Lo standards to cauterize the ewes as not pregnant, recheck, or pregnant.

Statistical Analysis: Data generated were analyzed using descriptive statistics and presented in tables. Sensitivity and specificity of the traditional post slaughter examination was analyzed using Graph Pad [29], Kappa statistics was also used to determine the measure of agreement between the two tests.

RESULTS

The breed of sheep sampled were Ouda, Balami, Yankasa and their crosses with weights ranging from 12.9 to 47.2 Kg. The pregnancy status of ewes slaughtered is presented in Table 1. Out of a total of 67 ewes slaughtered, 27 (40.3%) were detected pregnant by PSPB assay, while 10 (14.9%) were pregnant by post slaughter examination. Table 2 shows the comparison between the two diagnostic tests in detecting pregnant ewes at slaughter. Assuming PSPB was the gold standard, it was 100 % sensitive while, post slaughter was 37 % sensitive. The specificity was 100% and 85.1%, for PSPB and post slaughter examination, respectively. PSPB was 100 % accurate, while post slaughter was 74.6 % accurate in detecting pregnancy at slaughter. The kappa value for the measure of agreement between the two diagnostic tests was 0.41.

Table 1: Pregnancy status of ewes slaughtered in Sokoto, Nigeria (n = 67)

Diagnostic Test	Frequency	Prevalence (%)
Assay for pregnancy specific protein-B	27	40.3
Post slaughter examination	10	14.9

Table 2: Comparison of pregnancy specific protein-B and post slaughter examination in detecting pregnancy of ewes at slaughter

	Sensitivity	Specificity	Accuracy	Kappa value
PSPB	100.0%	100.0%	100.0%	0.41
Post slaughter	37.0%	85.1%	74.6%	

P < 0.0001, Confidence interval – 95%

Based on the assumption of PSPB assay as gold standard

PSPB - Pregnancy specific protein-B

Table 3: Sex of foetus and stage of pregnancy of slaughtered ewes in Sokoto by post slaughter examination (n =11)

	Number	Percentage (%)
Sex		
Male	4	36.4)
Female	7	63.6
Stage of pregnancy		
First trimester	2	18.2
Second trimester	8	72.7
Third trimester	1	9.1

Note: One ewe with twins at second trimester was recovered at post slaughter examination

Table 4: Categorization of assay for pregnancy specific protein-B results in ewes at slaughter

Category	Number	Percentage (%)
Pregnant	27	46.3
Not pregnant	31	13.4
Recheck	9	40.3

The sex and stage of pregnancy of ewes slaughtered is presented in table 3. Out of 11 (10 singleton and 1 twin) foetuses recovered at post slaughter examination, 4(36.4%) were males, while 7 (63.6%) were females. Based on stage of pregnancy; post slaughter examination revealed 2 (18.2%) foetuses were in first trimester, 8(72.7%) in second trimester and 1 (9.1%) in third trimester. Table 4 shows the categorization of test results for PSPB assay of ewes at slaughter. A total of 27 (40.3%) ewes were detected pregnant, 31 (46.3%) were not pregnant and 9 (13.4%) required to be rechecked.

DISCUSSION

The pregnancy status of slaughtered ewes detected by assaying for pregnancy specific protein-B (PSPB) was 40.3%. This is higher than the 14.9% detected during post slaughter examination in the same animals. Post slaughter examination involved visual inspection, palpation and incision of the uteri of slaughtered ewes, which relies on the presence or absence of foetuses from the uterine horns. This method is flawed by its inability to detect early pregnancy particularly during the embryo stage before organogenesis, in which no foetus is present. It is possible that several ewes in this stage of pregnancy were passed during post slaughter examination as non-pregnant thereby leading to pregnancy wastage. Previous studies revealed that pregnancy detected at slaughter of ewes ranged from 16.5% [12] to 50.2% [21]. However, this may not reflect the true pregnancy status of ewes in these slaughter houses. PSPB detected all the ewes diagnosed pregnant by post slaughter examination. The assay had a better ability to identify true pregnant and non-pregnant ewes than post slaughter examination, thereby proving to be more accurate.

PSPB is produced by the developing trophoblast and released into circulation where their concentrations can be detected and used to investigate placental function [23]. Its concentrations are detectable in pregnant ewes by day 18 of gestation, while they are low or undetectable in non-pregnant ewes [30]. Following fertilization in the sheep, the embryo periods begins and extends till about 30 - 40 days [3] after which foetal period begins and extends till parturition [5]. Post slaughter examination can only detect pregnancy after day 40, unlike PSPB that is capable of detecting pregnancy as early as 18 days post breeding [30].

Based on post slaughter examination of the recovered foetuses, there was a female predominance, with a female to male ratio of 1.8:1. The sex of pregnancy established by

PSPB could not be determined. This is expected as the test was not designed to determine sex but detect pregnancy. The study also showed that majority of the pregnant ewes slaughtered were in their second trimester of pregnancy. PSPB revealed that seventeen other ewes slaughtered were pregnant but undetected by post slaughter examination. These ewes may have been pregnant in their embryonic stage of the first trimester when the foetus is yet to be formed, suggesting that first trimester pregnancy was actually more than other trimesters.

The PSPB assay showed that about 13.4% of ewes sampled required their PSPB assay rechecked, while others were either pregnant or not. This category of ewes with unknown status was not available for re-evaluation since they had been slaughtered. However, using PSPB as the method of pregnancy detection would have slaughter decision deferred for such ewes, making them available for re-assessment.

CONCLUSION

The study shows that assay for pregnancy specific protein-B is a better method than post slaughter examination for evaluating pregnancy status of ewes prior to slaughter. The test is more sensitive, specific and accurate in detecting the pregnancy status of ewes for slaughter. The use of the traditional method of post slaughter examination alone leads to slaughter of substantially high number of pregnant ewes particularly those without foetuses in their uterus. It is recommended that assay for PSPB should be included in the routine ante mortem examination of ewes prior to slaughter. This will provide for deferred decision on ewes whose status requires recheck. The bioPRYN flex used in this study is manufactured for detecting PSPB in cattle, small ruminant and bison/buffalo. We therefore recommend that similar studies be carried out using these other animals to ascertain their true pregnancy status at slaughter.

ACKNOWLEDGEMENT

The authors appreciate the Animal sellers and butchers association of Bassa-shuni slaughter slab for their cooperation. The technical assistance rendered by Mr A. B. Shuaibu during Laboratory analysis is also appreciated.

Conflict of Interest Statement: The authors declare that they have no conflict of interest.

REFERENCES

1. Blench, R.M., 1999. Traditional livestock breeds: geographical distribution and dynamics in relation to the ecologys of West Africa. Retrieved from http://www.odi.org.uk/resources/download/2041.pdf.
2. Umaru, M.A., A.A. Adeyeye, A. Abubakar and H.S. Garba, 2009. Retrospective analysis of reproductive cases of domestic ruminant animals in Sokoto, Nigeria. Animal Research International, 6(1): 946-948.
3. Jainudeen, M.R. and E.S.E. Hafez, 2000. Gestation, Prenatal Physiology and Parturition. In: Reproduction in Farm Animals, Hafez, E.S. E and Hafez, B. eds., John Wiley and Sons, pp: 140-155.
4. Noakes, D.E., T.J. Parkinson and G.C.W. England, 2001. Arthur`s Veterinary Reproduction and Obstetrics. 8th edition, Elsevier, Sannders, pp: 107.
5. Abassa, K.P., 1995. Reproductive losses in small ruminants in Sub-Saharan Africa: A Review.
6. Alhaji, N.B., F.O. Fasina, M.S. Abubakar, I.A. Muraina, U.M. Chafe, A. Shittu, H.S. Lee, J. Onyango and I.A. Odetokun, 2015. Time-series analysis of ruminant foetal wastage at a slaughterhouse in North Central Nigeria between 2001 and 2012. Onderstepoort Journal of Veterinary Research, 82(1): 1-13.
7. Swai, E.S., A.A. Hayghaimo, A.A. Hassan and B.S. Mhina, 2015. The slaughter of increased numbers of pregnant cows in Tanga abattoir, Tanzania: A cause for concern?`. Onderstepoort Journal of Veterinary Research, 82(1): 1-5.
8. Nwakpu, P. and I. Osakwe, 2007. Trends in volume and magnitude of foetal waste of slaughter animals (2000-2005) in Ebonyi State of Nigeria. Research Journal of Animal Science, 1(1): 30-35.
9. Muhammad, I.R., R. Ashiru and A.Y. Abdullahi, 2007. Implications of the slaughter of Pregnant ewes and does to future stock in the Semi-arid Urban Abattoir. Journal of Animal and Veterinary Advances, 6: 819-822.
10. Addass, P.A., A. Midau, M. Milka and M.A. Tizhe, 2010. Assessment of abattoir foetal wastage of cattle, sheep and goat in Mubi main abattoir, Adamawa State, Nigeria. World Journal of Agricultural Science, 6(2): 132-137.
11. Bokko, P.B., 2011. Pregnancy wastage in sheep and goat in the Sahel region of Nigeria. Nigerian Veterinary Journal, 32(2): 120-126.

12. Alhaji, N.B. and I.A. Odetokun, 2013. Food security and economic implications of small ruminant fetal wastages in Nigeria: a case of an abattoir. Livestock Research for Rural Development, 25(5).
13. Akpabio, U. and S. Babalola, 2014. Incidence of foetal wastage and its economic implications in cattle slaughtered at Abak slaughterhouse, Abak, Akwa-Ibom State`. J. Reprod. Infertility, 5(3): 65-68.
14. Ogunbodede, M.A. and G.M. Oladele, 2016. Wastage of bovine conceptus through indiscriminate slaughter of pregnant cows at Bodija central abattoir, Ibadan. Journal of Agriculture and Crop Research, 4(4): 60-65.
15. Raimi, C.O., B.O. Oduguwa and F.O. Bamgboye, 2017. Slaughtered cattle and reasons for slaughtering of cows in ember months at Lafenwa abattoir in Abeokuta, Nigeria. European Journal of Agriculture and Forestry Research, 5: 1-8.
16. Amuta, P.O., K.A. Tordue, C.A. Kudi and L.I. Mhomga, 2018. Economic implication of foetal wastages through slaughter of pregnant pigs: A case study of the Makurdi Municipal Abattoir in Benue state, Nigeria. Asian Journal of Research in Animal and Veterinary Sciences, 1(2): 1-8.
17. Okorie-Kanu, O.J., E.V. Ezenduka, C.O. Okorie-Kanu, C.O. Anyaoha, C.A. Attah, T.E. Ejiofor and S.O. Onwumere-Idolor, 2018. Slaughter of pregnant goats for meat at Nsukka slaughter house and its economic implications: A public health concern. Veterinary World, 11(8): 1139-1144.
18. Atawalna, J., B.O. Emikpe, E. Shuaibu, A. Mensah, O.D. Eyarefe and R.D. Folitse, 2013. Incidence of fetal wastages in cattle slaughtered at Kumasi Abattoir, Kumasi, Ghana. Global Veterinaria, 11(4): 399-402.
19. Zulu, V.C., A.M. Mwanza, F.C. Banda, J. Yasuda and M.Y. Oshida, 2013. Cattle reproductive wastage in Zambia: a case of Mongu abattoir. Bulletin of Faculty of Agriculture Kagoshima University, 63: 49-54.
20. Jarikre, T.A., B.O. Emikpe, R.D. Folitse, T.K. Odoom, A. Fuseini and E. Shaibu, 2014. Assessment of fetal wastage in cattle, goat and sheep slaughtered at tamale abattoir, northern region, Ghana. Bulletin of Animal Health and Production in Africa, 62(1): 31-35.
21. Tasiame, W., B. Emikpe, R.D. Folitse, C.O. Fofie, S. Johnson, V. Burimuah, J. Atawalna, E. Boateng and E. Amemor, 2016. Foetal wastage in sheep and goats at the Kumasi abattoir in Ghana: A cross sectional study. Archives of Basic and Applied Medicine, 4(3): 125-128.
22. Xie, S., B.G. Low, R.J. Nagel, J.F. Beckers and R.M. Roberts, 1994. A novel glycoprotein of the aspartic protease gene family expressed in bovine placental trophectoderm. Biology of Reproduction, 51(6): 1145-1153.
23. Sousa, N.M., Z. Beckers and I. Gajewski, 2008. Current trends in follow-up of trophoblastic function in ruminant species. Journal of Physiology and Pharmacology, 59(9): 65-74.
24. Karen, A., B.E. Amiri, J.F. Becker, J. Sulon, M.A. Taverne and O. Szenci, 2003. Comparison of accuracy of transabdominal ultrasonography, progesterone and pregnancy associated glycoprotein test for determination between single and multiple pregnancy in sheep. Theriogenology, 66 (2): 314-322.
25. Adeyeye, A.A., I.U. Ate, A.I. Lawal and S. Adamu, 2016. Changes in some pregnancy biomarkers of Yankasa ewes experimentally infected with Trypanosoma evansi`. Animal Reproduction Science, 167: 109-116.
26. Sowande, O.S. and O.S. Sobola, 2008. Body measurements of West African dwarf sheep as parameters for estimation of live weight. Tropical Animal Health and Production, 40(6): 433-439.
27. Adu, I.F. and L.O. Ngere, 1979. The indigenous sheep of Nigeria. World Review of Animal Production, 15(3): 51-62.
28. Sivachelvan, M.N., M.G. Ali and G.A. Chibuzo, 1996. Foetal age estimation in sheep and goats. Small Ruminant Research, 19(1): 69-76.
29. GraphPad, 2000. GraphPad InStat version 3.05 for Windows 95, GraphPad Software Inc., San Diego California USA, (www.graphpad.com).
30. El-Amiri, B., Y. Cognie, J. Sulon and A. Karen, 2003. Pregnancy-associated glycoprotein concentrations in plasma and milk samples for early pregnancy diagnosis in Lacaune dairy sheep. Reproduction in Domestic Animals, 38(4): 319-364.