Qualitative evaluation of early pregnancy using Rapid detection milk kit in Graded Murrah buffalo cows

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Abstract

"Heat-aid kit" is a rapid detection kit which was developed by the application of ELISA. This qualitative test was indicated by colour change i.e based on the presence of progesterone. The present research was carried out to evaluate the accuracy of heat aid kit, a rapid detection test for early pregnancy diagnosis in graded Murrah buffaloes. For this, the milk samples were collected from 16 buffaloes early in the morning on day 21 post insemination and were subjected to pregnancy diagnosis using this test. Development of dark/strong pink was considered as estrus and these animals were certainly non pregnant. If the colour was very pale/colourless, that the animal may be pregnant or in mid cycle and confirmation of pregnancy was done by rectal palpation on days 45-60 post insemination. It was concluded that, this test was 85.71 accurate in diagnosing pregnancy and 100 per cent in diagnosing non-pregnancy. The overall diagnostic accuracy, positive and negative predictive values of this test were 93.75, 100 and 90 per cent, respectively.

Key words: pregnancy diagnosis; murrah buffaloes; rectal palpation; milk progesterone; ELISA.

Introduction

Early pregnancy diagnosis is a key to shorten the calving interval through early identification of open animals and their timely treatment and rebreeding so as to maintain a postpartum barren interval close to 60 days. Fricke (2010) stated that for successful integration into a reproductive management system, an ideal early pregnancy test for dairy cattle should be sensitive, specific, inexpensive and simple to conduct under field conditions. Establishing the progesterone levels in milk or serum between days 18 and 24 post insemination is an immunological method, an indirect one for determining the pregnancy (Sasser and Ruder, 1987). The concentration of progesterone in blood and milk at 20-24 days post insemination has been used as a tool for an early diagnosis of pregnancy in cattle (Ginther *et al.,* 1976). Therefore, the present investigation was carried out to detect pregnant and non-pregnant animals using milk on day 21 post insemination.

Materials and methods

Sixteen inseminated she buffaloes presented to the department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Gannavaram, which did not return to estrus by day 19-21 were subjected to early pregnancy diagnosis using Heat-Aid Rapid detection milk kit on day 21 post insemination.

Principle of Heat-Aid kit: It is a qualitative test indicated by the colour change that is based on the presence of progesterone hormone in the milk at the time of testing and this is developed by employing highly sensitive technique of enzyme linked immunosorbent assay (ELISA).

Collection of milk samples: The milk samples from inseminated buffaloes were collected in a clean vessel after discarding first few strips (foremilk). Samples were tested immediately after collection and the milk sample was mixed thoroughly before testing.

Procedure: The test was undertaken on 21 post insemination using Heat aid kit (Polchem Hygiene Laboratories Pvt. Ltd, Pune). The test kit consists of 16 wells just like the 96 wells plate and each well was covered with aluminium foil. The foil lid from the required wells was opened with a sharp knife/razor blade and peeled off. Empty the liquid and tap the plate dry on a tissue paper. 3-4 drops of milk sample was added to each well to be tested using separate dropper supplied. Then 4-5 drops of reagent A was added to each well to fill it over half. The plate was placed at room temperature for an hour. The wells were emptied and washed three times with drinking water. At each time, the wells were washed, then tapped and waved to dry them. After this, 4-5 drops of reagent B was added to each well to fill it over half. Results could be interpreted within 10-20 minutes of addition of reagent B with change or no change of colour. Development of dark/strong pink was considered as estrus and these animals were certainly not pregnant. If the colour was very pale/colourless, that the animal may be pregnant or in mid cycle. Accuracy in the form of sensitivity, specificity, positive and negative predictive values of the present technique was calculated as per the formulas given by Pieterse *et al.* (1990).

Results and Discussion

Out of 16 buffaloes tested, 6 (37.5%) were diagnosed as pregnant upon observation of no colour change and 10 (62.5%) as non-pregnant upon appearance of pink colour (Fig. 1). Out of 10 buffaloes diagnosed non-pregnant by this test, 1 became pregnant upon rectal palpation at day 45-60 (Table. 1). One buffalo that was diagnosed as non-pregnant with appearance of pink colour has drawn to the prediction of non-pregnancy by this test at day 21 post insemination but subsequently it became pregnant on rectal palpation at day 45-60. The pregnancy and nonpregnancy status of these buffaloes was confirmed by rectal examination at day 45-60. One buffalo though diagnosed as nonpregnant using this kit at day 21 post insemination was found positive for pregnancy at day 45-60 on rectal examination.

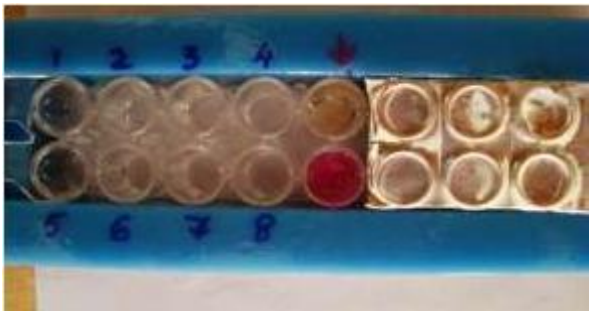

Fig. 2 Pink colored well indicating nonpregnancy and colorless well indicating pregnancy

As per the above mentioned observations in the present study, the sensitivity and specificity of pregnant and non-pregnant at day 21 post insemination was 85.71 and 100 per cent, respectively (Table. 1). The overall diagnostic accuracy in detecting early pregnancy using this kit at day 21 post insemination was 93.75 per cent (Table. 1). Further, the positive and negative predictive values were recorded as 100 and 90 per cent, respectively. The accuracy per cent of 85.71 in detecting early pregnancy in buffaloes using this commercial kit in the current study was comparable with the earlier findings of Kaker *et al.* (1993) in buffaloes, Ropstad and Refsdal (1985) and Stevenson and Call (1986) in cows, who reported 90.00, 88.6 and 84.00 per cent respectively using different on farm EIA based milk progesterone kits. The present finding (85.71 per cent) was much higher than the observations of Nebel *et al.* (1987) and Gupta *et al.* (1991), who recorded 69.7 and 71.43 per cent respectively.

Table. 1 Accuracy of pregnancy diagnosis using heat-aid kit versus rectal palpation (n=16).

S. N.	Diagnosis particulars	Diagnosis at day 21 post AI using heat-aid kit	Diagnosis by Rectal palpation (day 45-60)
1.	Diagnosis pregnant correct (a)	6/16	7/16
2.	Diagnosis pregnant incorrect (b)	0	0
3.	Diagnosis nonpregnant correct (c)	9	9
4.	Diagnosis nonpregnant incorrect (d)	1	0
5.	Sensitivity (Se ; %) 100 × a/(a+d)	85.71	100
6.	Specificity (Sp ; %) 100 × c/(c+b)	100	100
7.	Positive predictive value (PPV ; %) 100 × a/(a+b)	100	100
8.	Negative predictive value (NPV ; %) 100 × c/(c+d)	90.0	100
9.	Overall diagnostic accuracy	93.75	100

The accuracy of this kit in detecting non-pregnancy in buffaloes observed in the present study was 100 percent and these were in agreement to the findings of Singh and Puthiyandy (1980), Kaul and Prakash (1994) and Ucar *et al.* (2004) in buffaloes and Inaudi *et al.* (1982), Elmore (1986), Takeuchi *et al.* (1987) in cows who also reported 100 per cent accuracy. The above mentioned finding (100 per cent) was much higher than the observations of Gupta *et al.* (1991), Kaker *et al.* (1993) in buffaloes and Wimpy *et al.* (1986), Nebel *et al.* (1987) in cows, who reported 81.82, 79.6, 81.00 and 75.00 per cent, respectively.

It was concluded that the Heat-aid kit was 100 per cent accurate in diagnosing non-pregnancy and less accurate (85.71 per cent) in diagnosing early pregnancy. Hence, this type of ELISA based kit could be used for on farm pregnancy diagnosis for detecting open animals with 100 per cent reliability on day 21 post insemination which were in agreement to the findings of Rajamahendran*et al.* (1990).

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