

BOVINE PAPILLOMAVIRUS TYPE 2 IN REPRODUCTIVE TRACT AND GAMETES OF SLAUGHTERED BOVINE FEMALES

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ABSTRACT

Papillomaviruses are described selectively infecting epithelial tissues and are associated with many forms of cancer in different species. Considering the widespread dissemination of papillomatosis in livestock, interest is being centred on possible forms of viral transmission and respective mechanisms. In the present study, we report the detection of bovine papillomavirus (BPV) DNA sequences in female reproductive tract tissues, fluids and oocytes from slaughtered bovines not afflicted by cutaneous papillomatosis. BPV-2 DNA sequences were found in ovarian and uterine tissues as well as in oocytes, *cumulus* cells and uterine flushings. The presence of papillomavirus sequences in reproductive organ tissues and fluids shows that viral infection in organisms can be verified in others tissues, not only in epithelial ones. The present findings alert to the possibility of BPV transmission in embryo transfer programs and assisted fertilization procedures.

Key words: BPV-2, papillomatosis, gametes, PCR.

INTRODUCTION

The development of techniques applied to the bovine reproduction resulted in the necessity of control of the diseases of the reproductive tract, mainly infectious agents. It has been discussed about the possibility of transmission of these agents by embryo transfer (1,2,3). Yet, there are no data about papillomaviruses detection in tissues of bovine female reproductive tract and gametes. Bovine papillomaviruses are a small group of 6 different double strand DNA viruses that can infect fibroblasts and queratinocytes (types 1, 2 and 5) and epithelial cells (types 3, 4 and 6) (4). BPV-2 induces fibropapillomas in the head and neck of animals and is associated to natural lesions of the urinary bladder, although it may occasionally be related to fibropapillomas in the oesophagus and rumen (4). The bovine papillomatosis causes economic

losses spread in dairy farms in Brazil (5). The fact associated to the increased use of embryo transfer and *in vitro* fertilization techniques demands accurate studies to evaluate the presence of bovine papillomavirus in the female reproductive tract and gametes which is the aim of the present report. The viral type selected for this evaluation was BPV-2 due to its enlarged frequency in our previous data.

MATERIALS AND METHODS

We selected animals from Vale do Paraíba-SP, Brazil to confirm the high incidence of BPV-2 and eventual presence in the reproductive tract. We selected 3 bovine females (*Bos taurus taurus*) afflicted by cutaneous warts and proceeded the uterine flushing with a saline buffer (PBS) and surgical removal of wart samples. About 25mg of tissue and 200% of flushing fluid were

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used for DNA extraction by Quiagen columns according to respective protocol. About 300ng of the extracted DNA were then used for PCR analysis with specific primers to BPV-2 (Fw: 5'-GTTATACCACCCAAAGAAGACCCT-3'; Rev.: 5'-CTGGTTGCAACAGCTCTCTTTCTC-3'). PCR protocol was developed according to Stocco dos Santos *et al.* (6). After detection of BPV-2 sequences in the analysed samples, confirming the presence of papillomavirus in uterine environment, we collected the ovaries and segments of the uterus of 2 slaughtered females (*Bos taurus taurus*) not showing cutaneous warts. These animals came from a herd with a high incidence of papillomatosis. The ovarium follicles were aspirated to pick up the oocytes and then the oocytes were separated from *cumulus* cells by pipetting. DNA extraction from ovaries, uterine samples, oocytes and their respective *cumulus* was performed as previously described for warts and uterine flushings. PCR was made in the same conditions using the same specific primers to BPV-2 and primers to bovine β -globin (Fw: 5' -AACCTCTTTGTTTACAACCAG-3'; Rev: 5' -CAGATGCTTAACCCACTGAGC-3') to evaluate the DNA conditions for PCR. Confirmation of PCR results was obtained by southern blot using BPV-2 genome as probe.

RESULTS

The method described was efficient to detect BPV-2 DNA in the samples investigated as showed in Fig. 1A and 1B. One of the 2 slaughtered females did not show positive detection of BPV-2 in the uterus and ovaries samples.

DISCUSSION

In this study we report the presence of papillomavirus in the reproductive tract and gametes of bovine females. Although the vast literature concerning the contamination of the uterus and gametes of bovines by viruses as bluetongue virus (BTV), bovine leukaemia (BLV) and virus of the bovine viral diarrhoea (BVDV) (7,8,9), this is the first report describing the presence of papillomavirus in these tissues and cells. Vanroose *et al.* (10) suggested that the viruses can get the oocytes through granulosa and *cumulus* cells although they have not discussed how the viruses can reach these cells. Stocco dos Santos *et al.* (6) demonstrated the presence of papillomavirus in the blood of naturally infected animals. The presence of viruses in the bloodstream, theoretically allows them to be carried to any irrigated tissues. This possibility could explain the presence of BPV-2 in the ovaries, uterus, *cumulus* and oocytes of the investigated animals. Papillomaviruses are discussed as epitheliotropic (4) but the presence of DNA of papillomavirus in reproductive organ tissues, fluids and gametes indicates that viral infection could not be so specific. Many authors have been discussed the possibility of transmission of infectious agents by embryo transfer

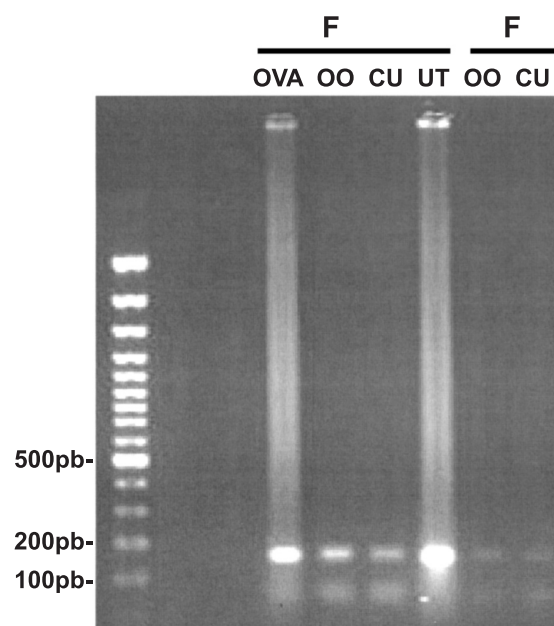


Figure 1A. BPV-2 DNA sequences detected in the samples of ovaries (OVA), uterus (UT), oocytes (OO) and *cumulus* (CU) from one of the two slaughtered cows and samples of oocytes (OO) and *cumulus* (CU) from the other female.

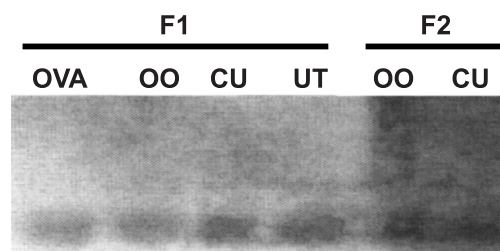


Figure 1B. Confirmation of the results by southern blot with specific probes to BPV-2.

and *in vitro* fertilization procedures and the indispensable control of the cells related to the procedures (1,2,3,11). Our data emphasize the need to include papillomaviruses as important agents to be investigated in reproduction procedures with rigorous control of uterine environment of embryo donors, recipients and oocytes collected from slaughtered animals.

RESUMO

Detecção de papilomavírus bovino tipo 2 em trato reprodutivo e gametas de fêmeas bovinas

Os vírus do papiloma bovino, descritos como agentes infectantes específicos do epitélio, têm sido associados a

diversas formas de câncer em diferentes espécies animais. Dada a intensa disseminação da papilomatose nos rebanhos, a investigação de diferentes formas de transmissão e seus respectivos mecanismos tem exigido especial atenção. No presente estudo, é relatada a detecção de seqüências genômicas do papilomavirus bovino (BPV) em ovócitos e tecidos do trato reprodutivo oriundos de fêmeas abatidas comercialmente, não apresentando papilomatose cutânea. A presença de DNA de BPV-2 em tecidos do trato reprodutivo, lavado uterino, ovócitos e células do *cumulus* traz evidências de que a infecção viral pode se desenvolver fora do tecido epitelial. Esses achados alertam para a possibilidade de transmissão do BPV através dos procedimentos de transferência de embriões e de fertilização *in vitro*.

Palavras-chave: BPV-2, papilomatose, gametas, PCR.

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