Cercetări Agronomice în Moldova
Vol. XLV , No. 2 (150) / 2012

DETECTION OF ORF VIRUS AND PAPILLOMAVIRUS OUT OF SAMPLES FROM GOATS AND CATTLE WHICH WERE GATHERED BY MULTIPLEX PCR ROMANIA

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Received February 1, 2012

ABSTRACT. The lesions which appear on the skin and are caused by parapoxvirus, herpesviruses and papillomavirus, depending on they're gravity, can produce large economic losses. The identification of viral species with different laboratory methods can facilitate making a correct diagnostic in identifying the infectious entity. The most common encountered injuries , especially on goats and kids, have an oral location, breasts, eyes and genital and podal. On the other side on cattles infected with papillomavirus the lesions are multiple and spread all over the body. To identify the etiologic agent from goats and cattle with skin lesions, tissue samples were collected and scabs. The virus identification was made with molecural biology techniques. The PCR method revealed the viral DNA, thus identifying a portion of the B2L preserved gene which is then amplified using primers PPP1-PPP4. For papillomavirus detection, primers FAP59 and FAP 64 were used to conserve the L1 human gene, primers that can help detect other types of papillomavirus from other species. The PCR results showed that lesions from goats were made by Orf virus, a virus that belongs to the papillomavirus gene, as for cattles the bovine papillomavirus. Studies have shown that PCR is a rapid laboratory method which can be used to identify the viral agent that causes severe skin lesions.

Key words: Viruses; PCR; Primers; Injuries.

REZUMAT. Detectarea virusului Orf şi a papilomavirusului din probele recoltate de la caprinele şi bovinele din Romania prin multiplex PCR. Leziunile care apar la nivelul pielii, determinate de parapoxvirusuri, herpesvirusuri şi papillomavirusuri, în funcție de gravitatatea lor, pot produce mari pierderi de natură economică. Identificarea speciilor virale prin diferite metode de laborator poate uşura, astfel, punerea unui diagnostic precis în identificarea entităților infecțioase. Cele

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mai frecvente leziuni întâlnite, în special la capre şi iezi, au localizare bucală, peribucală, mamară, oculară, podală şi genitală. În schimb, la bovinele infectate cu papillomavirus, leziunile sunt multiple şi dispersate pe tot corpul. Pentru identificarea agentului etiologic de la capre şi bovine cu leziuni la nivelul pielii s-au recoltat probe de țesut şi cruste. Identificarea virusului s-a făcut prin tehnici de biologie moleculară. ADN viral a fost pus în evidență prin metoda PCR, identificându-se, astfel, o porțiune a genei conservate B2L, amplificată, apoi, cu ajutorul primerilor PPP1 – PPP4. Pentru detectarea papillomavirusului s-au folosit primerii FAP59 şi FAP64, utilizați pentru conservarea genei L1 de tip uman, primeri cu care se pot detecta şi alte tipuri de papillomavirus de la alte specii. Rezultatele obținute în urma PCR-ului şi a secvențializării au demonstrat că leziunile de la capre au fost produse de virusul Orf , virus ce face parte din genul Parapoxvirus, iar la bovine de Bovine papillomavirus. Studiile au demonstrat că PCR este o metodă de laborator rapidă şi poate fi utilizată pentru identificarea agentului viral ce determină leziuni grave la nivelul pielii.

Cuvinte cheie: virusuri; PCR; primeri; leziuni.

INTRODUCTION

Papillomavirus and parapoxvirus are viruses that have double-stranded DNA structure with a strong impact on human and animal health (Damon, 2007; McFadden, 2005). The taxonomic parapoxviruses are included in the Parapoxvirus cathegory, *Poxiviridae* family with an affinity for epithelium and mucous issues, producing bladder-pustulo-crusty looking lesions, similar to those infections made by other viruses such as: the popular stomatisis virus for cattles (BPSV), Orf virus (ORFV) and Pseudocowpox virus (PSCV). However, the Papillomaviruses are included in the Papillomavirus taxonomic type; *Papillomaviridae* family that makes proliferative lesions, vegetative type with variable shapes and sizes and affinity for keratinocytes.

The transmission of the two taxonimoc groups of viruses on humans and animals can be achieved either directly or indirectly because both animals and humans are carriers and they eliminate germs. The different diagnostics between infections made on one hand by papillomaviruses and on the other by parapoxviruses, can be made by using serological tests, virological, histological, and molecular biology methods.

Currently for detecting papillomaviruses (HPV) and parapoxviruses (PPV) the multiplex PCR method is being used. Since the literature has few information regarding the techniques used to distinguish the infections caused by the two taxonomic virus groups, the researches that followed showed the PCR multiplex method to classify the diagnostics.

MATERIAL AND METHODS

To detect both groups of taxonomic viruses, the extraction of viral DNA from collected tissues and scabs from six sick animals was used (three bovines infected with HPV and three goats infected with

PPV). The collected DNA was subject to PCR multiplex reaction. This way different combinations were made out of the two extracted DNA from the from the isolated viruses, to see if there is a possibility of a gene recombination and the chance that a new recombined strain might occur.To identify a portion of the B2L gene (592bp) which is specific to parapoxviruses, primers PPP1 (GTCGTCCACGATGAGCAG) and PPP4 (TACGTGGGAAGCGCCTCGCT) were used and for papillomaviruses primers FAP 59 (TAACWGTIGGICAYCCWTATT) and FAP64 (TACGTGGGAAGCGCCTCGCT), this primers being commonly used in human medicine to identify L1 gene with a lengh of 400 bp isolated from human HPV *(Table 1).*

Table 1 - Mix prepared to carry out multiplex PCR

Buffer 10X	5µl	
Sol Q	10µl	
dNTPs 10mM	8µl	
PPP1 (primer foward)	1µl	5’ GTCGTCCACGATGAGCAGCT 3’
PPP4 (primer revers)	1µl	5’ TACGTGGGAAGCGCCTCGCT 3’
FAP 59 (primer foward)	1µl	5’ TAACWGTIGGICAYCCWTATT 3’
FAP 64 (primer revers)	1µl	5’ CCWATATCWVHCATITCICCATC 3’
Taq	1µl	
H2O	17µl	
DNA	5µl	

Viral DNA extraction was performed using the NucleoSpin ® Tissue kit (Macherey - Nagel, USA).The final mix was subject to a 94 degrees distortion for 10 minutes followed by 45 amplification cycles, each cycle followed by a distorsion phase at 94 ^{0} for 1 minute and 30 seconds, an adjustement phase to 50 ^{0} for 1 minute and 30 seconds and an elongation phase at 72 ° C for 1 minute. At the end of the 45 cycles, the mix was subject to a final extension for 6 minutes at 72 ° C. The final amplified PCR multiplex was then subject to a electrophoresis analasys on 2% agarose gel (100mA, 400V). Each gel was loaded with 5 µl –amplified and 1 µl blue bromine – phenol. DNA broadening was then revealed by ethidium bromide gel by viewing it at translunimal (UV). For each electrophoretic type, known quantity with a molecular weight (100bp DNA Ladder BioLabs, New England) markers were used, negative control as well. Images were viewed with a Vision Works ® LS software, image analyzer attached to GelDoc-It310 (UVP, UK). The purification of the amplifiers obtained by PCR was performed using the NucleoSpin ® Extract II kit (Macherey-Nagel, USA) intended to eliminate the amplified primers, nucleotides and other substances used for PCR mix preparation.

RESULTS AND DISCUSSION

Multiplex-PCR results obtained after the extraction of viral DNA in tissue samples taken from six animals (three cattle infected with papilloma virus and three infected goats with parapoxvirus), confirmed the diagnosis, showing that this technique is useful in differentiating the two diagnoses *(Fig. 1).*

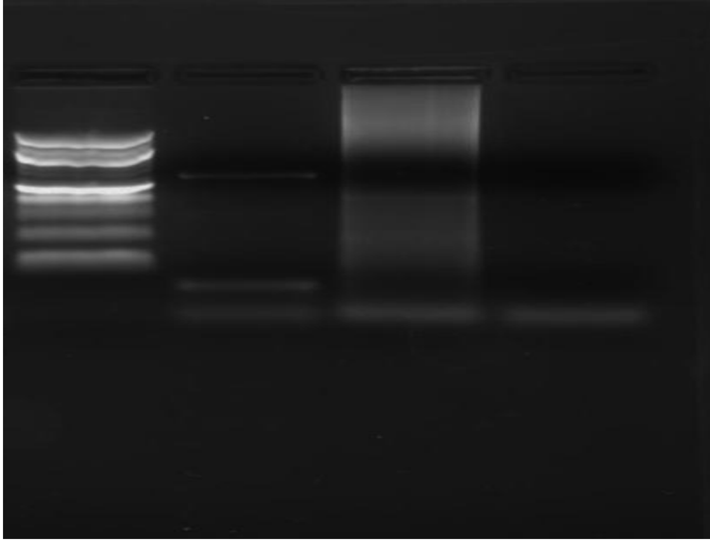

Figure 1 - HPV/PPV nested-multiplex standardization. The nested-multiplex was able to detect HPV and PPV DNA.

CONCLUSIONS

The PCR multiplex is a quick method to confirm laboratory diagnosis even if the anatomoclinical aspect is characteristic to HPV and PPV infections.

The method is recommended for a correct identification of the pathogen agent, the gene amplification being carried by a simultaneous co-infection of the extracted DNA from samples taken out of infected animals with two types of viruses.

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