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Colorimetric detection of caprine arthritis encephalitis virus (CAEV) through loop-mediated isothermal amplification (LAMP) with gold nanoprobes

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ABSTRACT

Infectious diseases in goats, CAE in particular have widely affected the productivity of this animal and greatly affected the farmers and the small ruminant industry. Molecular technique such as LAMP has been applied to detect the CAEV proviral DNA specifically and sensitively but this technique has its own drawback. Gold is the most widely used nanoparticle (NP) which has excellent properties such as high surface area and compatibility with biomolecules. In this study, gold nanoparticle (AuNP) conjugated with modified oligonucleotides or gold nanoprobe (AuPr) was used to detect CAEV proviral DNA in LAMP product. The hybridization of AuPr to a complementary sequence on the LAMP product made the gold resistant to high salt concentration. This resistance or non-resistance of the AuNP is observed as color change in the mixture. This study, reported a simple method of visual detection of CAEV proviral DNA in LAMP product using AuPr.

1. Introduction

Caprine arthritis encephalitis virus (CAEV) infection is one of the economically important diseases of goats. This virus is classified as a Small Ruminant Lentivirus (SRLV) together with Maedi Visna Virus (MVV), under the family Retroviridae (Abelson and Schoborg, 2003; Brinkhof et al., 2008; CFSPH, 2007; L'Homme et al., 2011). It causes a persistent disease which is characterized by polyarthritis in adult and progressive paresis (leukoencephalomyelitis) in kids (CFSPH, 2007; Clements and Zink, 1996; OIE, 2008; Smith and Sherman, 2009). Other clinical manifestations of CAEV infection includes interstitial pneumonia, mastitis, and chronic wasting diseases (Ali Al Ahmad et al., 2012; CFSPH, 2007; L'Homme et al., 2011). Most CAEV infections are subclinical in nature (APHIS, 2008; CFSPH, 2007). The CAEV is species specific, but recent research findings showed evidence of cross infection in sheep (Leroux et al., 1997; Pisoni et al., 2007, 2005; Santry et al., 2013; Shah et al., 2004).

The detection of CAEV infection is mainly done through serological tests such as agar gel immune-diffusion (AGID) test and enzyme-linked immuno-sorbent assay (ELISA) (Herrmann-Hoesing, 2010; OIE, 2008). Application of molecular methods such as polymerase chain reaction (PCR) has become a routine diagnostic procedure in the laboratory, since the rapidity of the assay aid in detection of CAEV even before antibody is detected using serological techniques (Brinkhof et al., 2008; CFSPH, 2007). Recently, loop-mediated isothermal amplification (LAMP) technique was optimized (Huang et al., 2012; Balbin et al., 2014) for the detection of CAEV proviral region. The optimized protocols were determined to be sensitive and specific. Though new techniques have been developed and used nowadays to detect various infectious diseases in livestock animals, still, most of the diseases are not being diagnosed properly. Therefore, continuous development of methods

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that are sensitive and specific and less expensive is needed (Kricka, 1999).

Nanotechnology is an emerging science which deals mainly with the study of matter with one or more dimension in between 1–100 nm. Gold nanoparticles (AuNP), also known as colloidal gold or gold colloids, are considered to be the most stable metal nanoparticles (NP). They present unique characteristics such as the behavior of the individual particles, size-related electronic, magnetic and optical properties, and compatibility with biomolecules (Daniel and Astruc, 2004). These properties have attracted researchers especially in the medical field to further explore this property of AuNP and apply in point of care disease diagnosis. The AuNP have been used specially in the development of different disease detection/screening platforms making it the most widely used NP (Ahmadpour-Yazdi et al., 2015; Castañeda et al., 2007; Khan et al., 2014).

In 1996, it was first shown that the optical properties of oligonucleotide functionalized AuNP, now called gold nanoprobe (AuPr) could be used in colorimetric assays for DNA detection (Mirkin et al., 1996). Further studies have been conducted using AuPr for the detection of pathogenic DNA and single nucleotide polymorphisms (SNPs) (Doria et al., 2010). Current researches showed favorable results in terms of the sensitivity and specificity of the method. The AuPr could detect relatively small amount of complimentary DNA, as low as ~54 ng (Bakthavathsalam et al., 2012). The AuPr can also detect point mutation in certain unamplified sequence within minutes without the use of complicated laboratory equipment and procedures (Bao et al., 2004). The discovery of the applications of NP in disease diagnosis allowed detection of pathogens, metabolites and even genes of medical importance in humans and in animals (Schmitt and Henderson, 2005; Sin et al., 2014).

Regular screening of animals from CAEV infection is one of the recommended management schemes to prevent the transfer and further spread of the disease. The LAMP technique, although considered as a simple molecular tool in the detection of different diseases is being limited by some factors which reduces its acceptability in clinical and laboratory setting (Todd Denison and Xiaokang, 2008). The color change in LAMP assay can result to false positive because of the reaction of SYBR green dye to magnesium ion present in the LAMP product. Therefore, this study was conducted to evaluate AuPr for the colorimetric detection of CAEV proviral DNA after the LAMP assay. The hybridization of AuPr to a complementary sequence on the LAMP product can make the AuNP resistant to high salt concentration. This resistance or non-resistance of the AuNP can be observed as color change in the mixture. Using this method, the drawbacks in LAMP can be overcome.

2. Materials and methods

2.1. Blood collection and DNA extraction

Blood samples were collected from different farms with reported cases of CAEV infection as confirmed by ELISA and nested PCR. The blood samples were collected in the jugular vein of each animal using a sterile needle and a heparinized vacutainer tube. All animals were handled by veterinarians from the Philippine Carabao Center (PCC) in accordance with good animal practice following the PCC institutional guidelines on Institutional Animal Care and Use Committee (IACUC) and on the Ethics of Animals for Research.

For the DNA extraction, briefly, the samples were centrifuged at 4000 rpm for 5 min. After centrifugation, 500 μL of the buffy coat was recovered and transferred to a sterile 1.5 mL microcentrifuge tubes. The DNA was then extracted using Wizard Genomic DNA Extraction Kit (Promega, USA) following manufacturer's instructions. The quality of the extracted DNA was determined through amplification of the β-actin gene amplification, a housekeeping gene. Only samples positive for β-actin gene was further subjected to LAMP assay.

Table 1
LAMP primers and modified oligonucleotide (thiol- labeled) used in the study.

Name	Sequence (3′-5′)
F3	ATGAAGAGGCGGAAAGGT
B3	GTTTGTGAGCCATATGCC
FIP	CCTACTCCCATAATTTGATCCACT-GGGAGAAGGAATAATCCACCA
BIP	GCACAGGGCTAACATGGATCAA-GACTGCTCTTAAGGCTGTT
F-Loop	TTAATCCTCCCCCCTGCCG
B-Loop	AGGCAGACATGCCTGGG
CAEV-RP	Thiol-CTTAATTGTCACCTAGTT

2.2. LAMP primers and thiol modified oligonucleotide

The primers were adopted from the previously published research (Balbin et al., 2014) which target the proviral gag gene, a relatively well conserved region of the SRLV, and an ideal region for primer design (Balbin et al., 2014; L'Homme et al., 2011). The thiol modified oligonucleotide was designed based on the CAEV sequence recently reported in the Philippines (Padiernos et al., 2015). The primers and modified oligonucleotide (Fig. 1) were synthesized by AIT Biotech (Singapore) and International DNA Technologies (USA), respectively (Table 1).

2.3. Detection of CAEV using LAMP assay

Amplification of CAEV DNA was performed following the previously published LAMP protocol (Balbin et al., 2014). The LAMP reaction was incubated at an isothermal temperature of 63 °C for 30 min. The result of the amplification was determined by direct observation of a color change in the LAMP reaction after addition of 0.5 μL of 10× SYBR Green dye (Invitrogen, USA). The LAMP product was also subjected to agarose gel electrophoresis to confirm the presence of characteristic multiple ladder-like bands pattern.

2.4. Synthesis of AuNP

In a clean 500 mL erlenmeyer flask, 10 mL of 25 mM Gold (III) tetrachloro hydrate was mixed with 240 mL of sterile ultrapure water, placed on a hotplate and left to boil. The mixture was then added with 25 mL of 38.8 mM Trisodium Citrate. Color change in the mixture from yellowish to wine red can be observed which indicate reduction of gold ions (Au^{3+}) to gold atoms (Au^{0}). Boiling and stirring was continued for 15 min and was then allowed to cool in room temperature. The volume of the solution was then adjusted to 250 mL using sterile ultrapure water and stored at 4 °C until used (Hill and Mirkin, 2006).

2.5. Functionalization of AuNP with modified oligonucleotide

The simplified protocol in the functionalization of AuNP with modified oligonucleotide was based on a previously described method (Zhang et al., 2012). Briefly, 1000 μL of AuNP was transferred in a sterile 1.5 mL microcentrifuge tube and was added with 10 μL of 100uM thiol modified oligonucleotide. The mixture was vortexed before addition of 101 μL of 100 mM Sodium Citrate-HCl then was mixed thoroughly. The pH of the solution was gradually adjusted to three (3) using 1.2 M HCl. The mixture centrifuged at 12500 rpm for 15 min and the supernatant was discarded. The AuNP was added with 1000 μL of 0.1 M PBS (pH 7) centrifuged at 12500 rpm for 15 min and the supernatant was discarded. This step