Molecular and Cellular Probes 30 (2016) 174–177

Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr

Short communication

Loop-mediated isothermal amplification assay for rapid and sensitive detection of sheep pox and goat pox viruses in clinical samples

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ARTICLE INFO

Article history:
Received 20 December 2015
Received in revised form
6 February 2016
Accepted 7 February 2016
Available online 10 February 2016

Keywords:
Sheep pox virus
Goat pox virus
LAMP
Visual detection
Field diagnosis

ABSTRACT

A Loop-mediated isothermal amplification (LAMP) assay targeting the highly conserved *DNA polymerase gene* of capripox virus genome was developed and evaluated for rapid detection of sheep pox and goat pox viruses. The optimized LAMP assay is found specific and sensitive for amplification of target DNA with a diagnostic sensitivity and specificity of 96.6% and 100% respectively compared to quantitative PCR. The detection rate of LAMP, PCR and Q-PCR assays is found to be 81.5%, 67% and 83% respectively. This LAMP assay has the potential for rapid clinical diagnosis and surveillance of sheep pox and goat pox in field diagnostic laboratories.

Sheep pox and goat pox known as Capripox caused by sheep pox virus (SPPV) and goat pox virus (GTPV) respectively are one of the World Organization for Animal Health (OIE) notifiable and trans-boundary important diseases of sheep and goats [1]. In India, the disease is endemic and incurs huge economic loss to farming community [2]. Several PCR and quantitative PCR (QPCR) assays have been in use for the sensitive detection of CaPV DNA in field clinical samples [3–7]. However, they are not deployable in less equipped rural diagnostic settings and the loop mediated isothermal amplification (LAMP) assay [8] would be of choice over these assays. In this study, a LAMP assay targeting the *DNA polymerase* (DPO) gene of the CaPV genome was developed and evaluated by comparing to conventional PCR and QPCR assays. Several PCR/QPCR assays have been developed earlier targeting DPO gene to diagnose different animal pox viruses namely capripox virus (CaPV) [5], orf virus (ORFV) [9], CaPV/ORFV [6,7] and also buffalopox virus (BPXV)/camelpox virus (CMLV) [10].

The vaccine strain GTPV-Uttarkashi, at passage level 60, was used for optimization of the LAMP assay. In addition, GTPV (n = 04), SPPV (n = 07), ORFV (n = 04), BPXV (n = 12) and CMLV (n = 09) isolates were included in evaluation of the analytical sensitivity and specificity of developed assay. A total of two hundred clinical samples (n = 200) consisting of skin scab, swabs, blood and other tissues of sheep and goats from diverse geographical origin (Table) was used in evaluation of the LAMP assay. The clinical tissue samples homogenized as 10% (w/v) suspension and cell culture isolates were used for the extraction of total genomic DNA (gDNA) as per standard manufacturer's protocol (AuPrep™, Life technologies Pvt. Ltd., New Delhi, India) and stored at −20 °C until further use. The LAMP primers were designed targeting the conserved region of DPO gene in PrimerExplorer v4 online tool and located at 32163 to 32387 nucleotides with respect to GTPV G20-LKV strain (AY077836). The LAMP primers are F3: 5′-GAAGAAAAAGTATACCACCCTTA-3′ (23 bp); B3: 5′-TGTAAATCACTTTCTAACGATGA-3′ (23 bp); FIP: GTTCCCT TATTGATACGTTCAGGAA-GAATTC-GCAATCGAAGAAAAAGTATACCA (48 bp); BIP: CAAAACAAGGCTTTTGCAAATGTT-GAATTC-TTTCAACA CATACTTGTACAGAT (47 bp). The outer primers F3 and B3 were used in optimization of conventional PCR. The optimum LAMP reaction conditions were identified at different temperatures (60–66 °C) and time range (15–75 min) using gDNA from purified GTPV. The conventional PCR using F3 and B3 primers showed an amplification of 225 bp product at 53 °C and the product was cloned

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http://dx.doi.org/10.1016/j.mcp.2016.02.004
0890-8508/

Table
Comparative evaluation of CaPV-LAMP, QPCR and conventional PCR assays.

Clinical specimens from different geographical locations (State)	Species		Total samples	PCR		TaqMan QPCR		CaPV-LAMP	
	Sheep	Goat		Sheep	Goat	Sheep	Goat	Sheep	Goat
Andhra Pradesh	19	12	31	15	11	16	12	15	12
Gujarat	10	01	11	08	00	09	00	10	00
Himachal Pradesh	22	01	23	13	00	21	00	21	00
Jammu and Kashmir	10	01	11	08	00	09	00	09	00
Maharashtra	18	24	42	10	19	14	21	15	21
Punjab	02	02	04	2	0	2	00	02	00
Rajasthan	20	02	22	16	02	17	02	16	02
Tamil Nadu	02	03	05	02	02	02	02	02	02
Orissa	00	07	07	00	01	00	03	00	02
Uttar Pradesh	23	07	30	18	02	21	07	19	07
Uttarakhand	00	11	11	00	05	00	08	00	08
Others	00	03	03	00	00	00	00	00	00
Total	**126**	**74**	**200**	**92**	**42**	**111**	**55**	**109**	**54**
Percent Positivity				**67**		**83.0**		**81.5**	

into pGEM-T Easy vector (Promega, Madison, USA) and the copy number of plasmid DNA template was determined. In addition to agarose gel analysis, visual inspection of LAMP reaction by adding SYBR green I (Invitrogen, Carlsbad, CA) and hydroxyl naphthol blue (HNB) (Sigma Aldrich, St. Louis, MO) dyes at a final concentration of 100X and 120 μM respectively was also employed. The cross reactivity of the primers to related pox viruses namely ORFV, BPXV & CMLV and other viruses of sheep/goats namely Peste des petits ruminants virus (PPRV) and bluetongue virus (BTV) was ruled out. The specificity of the LAMP structure was further confirmed by

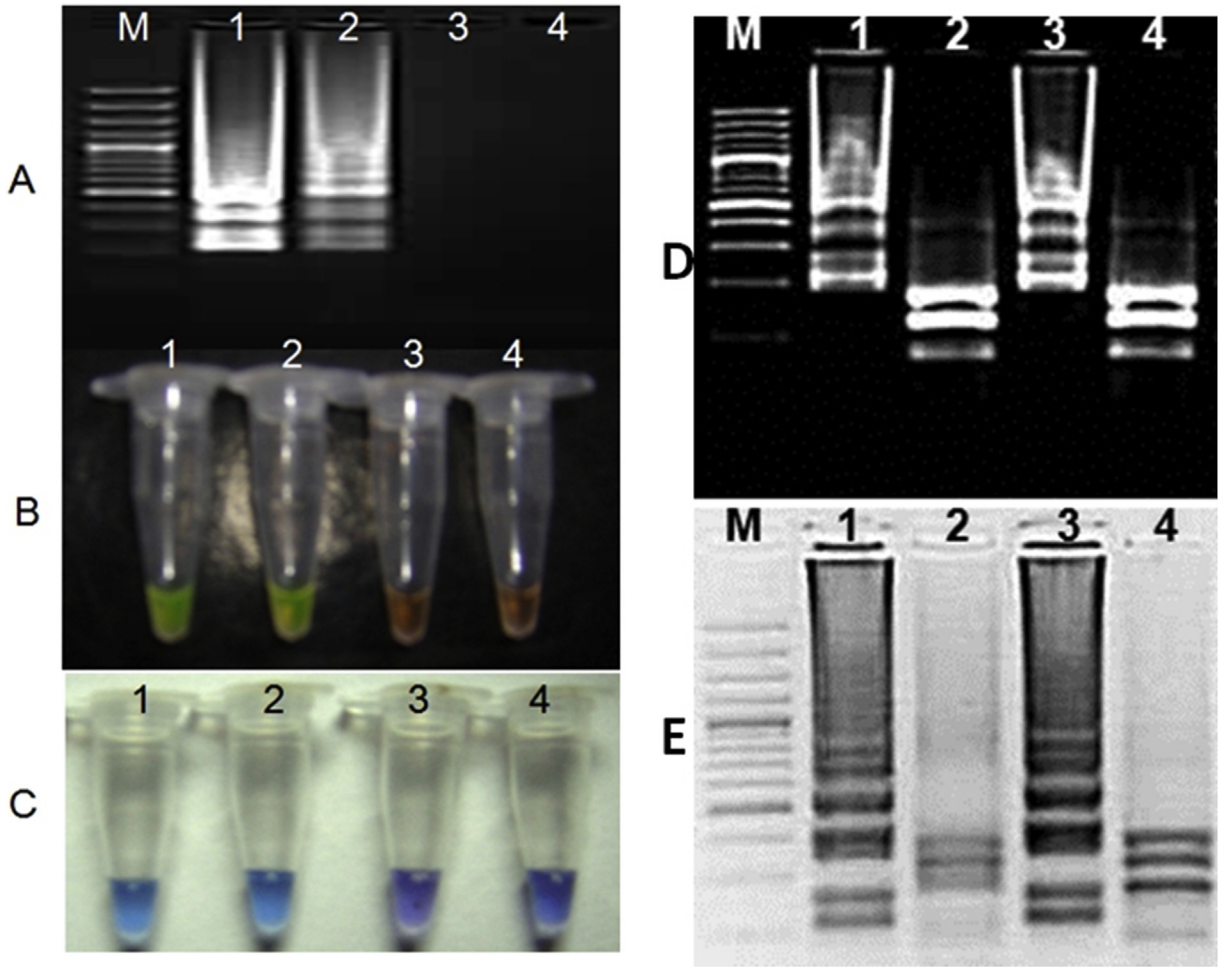

Fig. 1. Evaluation of specificity of CaPV LAMP assay (A) 2.5% agarose gel showing typical ladder-like pattern (Lane 1–2: GTPV-Uttarkashi and SPPV Srinagar 38/00) and no such amplification in negative controls (Lane: 3–4: ORFV Mukteswar 59/05 and NTC) (B) Specificity after addition of SYBR green I and (C) HNB dye (D) Restriction enzyme digestion of LAMP structure using EcoRI (Lane 2 & 4: digested products of SPPV and GTPV; Lane 1 & 3: respective undigested products) and (E) HinfI enzyme digestion (Lane 2 & 4: digested products of SPPV and GTPV; Lane 1 & 3: respective undigested products). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)