DOI: 10.2478/cjf-2024-0018

CODEN RIBAEG ISSN 1330-061X (print)
1848-0586 (online)

sciendo

APPLICATION OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION FOR *Edwardsiella ictaluri* DETECTION IN INDONESIA ENTERIC SEPTICEMIA OF CATFISH

Hidayati Sari Utami[1*], Rahman Rizky Amalia[1], Nainggolan Ronald[1], Ardiani Trisniaty[1], Asri Tina Yunia[1], Raden Wasito[2]

[1] *Fish Quarantine Standards Test Center, Fisheries Quality and Safety Control, Indonesia*

[2] *Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta 55281, Indonesia*

*Corresponding Author: azkafm@yahoo.com

ARTICLE INFO

Received: 1 March 2024
Accepted: 23 September 2024

Keywords:
Bacteria, catfish
Diagnostic method
Fish diseases
Molecular diagnosis

ABSTRACT

Loop-mediated isothermal amplification (LAMP) method is useful for rapidly detecting *Edwardsiella ictalurid* infection, especially enteric septicemia of catfish (ESC). This study aims to investigate the LAMP method for *E. ictaluri* detection in ESC. This research was an experimental study using a total of 55 catfish, consisting of 5 negative controls and 50 catfish injected intraperitoneally with 0.1 mL of *E. ictaluri* at a concentration of 105 CFU/mL. The kidneys of three fishes were randomly selected 6, 12, 18, 24, 30, 36, 42, 48, and 54 hours after infection with *E. ictaluri*. In addition, samples were also collected on days 3, 5, and 7. Bacterial analysis was determined by conventional biochemistry (genus and species test), whole-genome sequencing of catfish, and LAMP amplification. All types of data obtained in this study were analyzed by descriptive statistics to compare infected and healthy catfish. The catfish were infected with *E. ictaluri* after 12 hours of infection according to the LAMP amplification procedure which revealed hemorrhages throughout the body, fins, protruding eyes, necrosis, inflammation of the spleen, liver, intestine, pale gills, gut, abdomen swelling, and tissue necrosis in the upper part of the head. It can be concluded that the LAMP method is more effective than the PCR method for detecting infection with *E. ictaluri* in catfish.

How to Cite

Utami, H. S., Amalia, R. R., Ronald, N., Trisniaty, A., Yunia, A. T., Wasito, R. (2024): Application of loop-mediated isothermal amplification for *Edwardsiella ictaluri* detection in Indonesia enteric septicemia of catfish. Croatian Journal of Fisheries, 82, 145-152. DOI: 10.2478/cjf-2024-0018.

INTRODUCTION

Bacterial zoonotic diseases such as infections caused by *Aeromonas* spp., *Flavobacterium columnare*, and *Edwardsiella ictaluriis* lead to increased mortality in fish and reduce their nutritional quality (Wise et al., 2021). The infection of bacteria can be a primary infection and a secondary or co-infection through the environment, including sediment ponds and water (external factor), and host conditions (internal factor) (Halver and Hardy, 2002). *Edwardsiella ictaluri* is a gram-negative bacterium that causes disease in some animals, especially catfish. It is classified into Group 2 of Quarantine Pests in the fish aquaculture industry, according to the Decree of the Minister of Marine Affairs and Fisheries No. 91 of 2018. These bacteria are also pathogens in catfish known as enteric septicemia of catfish (ESC) with a mortality rate of up to 50%. They are widespread in the United States and Southeast Asia (Office International Des Epizooties, 2009). Due to ESC in the US, the industries were estimated to lose approximately $60 million annually (Office International Des Epizooties, 2009). Indonesian catfish or *Clarias* is the second most important freshwater aquaculture commodity after tilapia. *Clarias* is a species that can be easily cultured in areas with limited space and a lack of water resources (Gustiano et al., 2021). Furthermore, the data on infection with *Edwardsiella ictalurid* in Indonesian catfish farming is still limited. Therefore, preventing the spread of ESC has become a priority.

Loop-mediated isothermal amplification (LAMP) is a sensitive method and generates a deoxyribonucleic acid (DNA) copy of 109 CFU in less than one hour under isothermal conditions. It is applied for microorganism detection, including viral, bacterial, and parasitic pathogens. This method uses DNA polymerase I from Geobacillus stearothermophilus (also known as Bst DNAP) and four primers designed to recognize six target DNA sequences by four distinct sequences. A forward inner primer (FIP) contains DNA target sense and antisense sequence as the initial initiation for the LAMP. It is followed by the DNA strand displacement and an outer primer that releases a single-stranded DNA. Thus, the outer primer (F3) with a lower concentration conducts synthesis with DNA polymerase and produces single-stranded DNA. It serves as a template for DNA synthesis primed by the second inner and outer primers. Furthermore, it hybridizes to the other target and produces a DNA structure. The cycle continues to copy DNA and knot strands. The result can be seen by electrophoresis gel, dripping an amplification product with the SYBR Green I dye and observing it under UV light. A positive result produces a green glow, whereas a negative result remains orange. This method is expected to amplify the target sequence with high specificity (Savan et al., 2004).

Because of the high specificity, sensitivity, and reliability of these analyses, this research aimed to develop a rapid method for the identification of bacteria in catfish farming. Therefore, the objective of this research is to investigate the LAMP method for the detection of *E. ictaluri* in ESC.

MATERIALS AND METHODS

Research design

This was an experimental study with a total of 55 catfish from local breeders and culture around Jakarta, Indonesia, consisting of 5 catfish as negative control and 50 catfish (*Clarias*) injected intraperitoneally with 0.1 mL of *E. ictaluri* at a concentration of 105 CFU/mL. Acclimatization was carried out for one week prior to the treatment. The kidneys of three fishes were randomly selected 6, 12, 18, 24, 30, 36, 42, 48, and 54 hours after infection with *E. ictaluri*. In addition, samples were collected on days 3, 5, and 7.

Bacteria test

E. ictaluri bacteria were obtained from Stasiun Karantina Ikan Pengendalian Mutu (SKIPM), Mopah Merauke, originated from NCIMB 13272 and preserved by freeze-drying. The advantage of the freeze-drying method is that it is safer from the risk of degradation of compounds in the extract than the other methods. This is possible because a low temperature is used in this process (Muchtadi and Sugiono, 1992). Furthermore, bacteria were cultured in tryptic soy broth (TSB) media and incubated at 30 °C for 18 to 24 hours. Bacterial inoculation was determined by conventional biochemistry, PCR methods, and sequencing analysis before being tested on the fish.

Conventional biochemistry

E. ictaluri bacteria were inoculated into tryptic soy agar (TSA) media and incubated at 30°C for 24 hours. Subsequently, the two conventional tests were performed as follows:

- Genus test:
  Parameters of *E. ictaluri* genus test consist of gram stain, catalase test, oxidase, oxidation fermentation (OF) basal medium, ornithine, indole, motility, hydrogen sulfide (H_2S), and glucose (Holt et al., 2000).
- Species test:
  Parameters of *E. ictaluri* species test consist of arginine, Methyl Red (MR), Voges Proskauer (VP), urease, glycerol, xylose, gelatin hydrolysis, decarboxylase lysine, phenylalanine deaminase, aesculin hydrolysis, nitrate reduction, Simmon's citrate, mannitol, maltose, arabinose, lactose, salicin, trehalose, inositol, sucrose, melibiose, TSA, OF basal medium, sulphide indole motility (SIM), oxidase strip, catalase, McConkey, motility indole ornithine (MIO), gram stain, glucose, methyl red/voges-proskauer (MR/VP), lysine iron agar (LIA), kanamycin aesculin azide (KAA), Kovack reagents, nitrates.

Molecular analysis for isolate identification

Bacteria from TSB media were subjected to PCR analysis. The DNA inoculation was extracted by DNeasy blood and tissue kit from Qiagen. The DNA isolate was amplified by specific *E. ictaluri* primers: EictF [5'-ACTTATCGCCCTCGCAACTC-3'] and EictR [5'-CCTCTGATAAGTGGTTCTCG-3'] (Iwanowicz et al., 2009). The PCR products were visualized by agarose gel electrophoresis stained by SYBR Safe Green dye. The PCR products were sent to Makrogen (South Korea) for sequencing analysis and the results were aligned with two gene references (accession number: CP001600.1; AF110153.1).

Fish sample and treatment

Fish were infected by intraperitoneal injection of *E. ictaluri* with a standardized concentration of 150 CFU/ml. Catfish were injected intraperitoneally with 0.1 mL of *E. ictaluri* (bacterial density of 105 CFU/mL). Samples (liver and kidneys) were collected 6, 12, 18, 24, 30, 36, 42, 48, and 54 hours after infection with *E. ictaluri*.

Loop-mediated isothermal amplification (LAMP) analysis

Whole-genome sequencing of catfish

The DNA sequencing from catfish liver and kidneys was conducted using commercial kits (DNeasy blood and tissue kit, Qiagen). First, twenty-five milligrams of the organ were crushed into a 1.5 ml microtube. Then, the sample was added with 180 µL ATL buffer and 20 µL K proteinase, followed by a gentle vortex. The homogenate was incubated (56 °C for 1 to 3 hours), followed by mixing gently 2 to 3 times each hour. After incubation, it was vortexed for 15 seconds, added again with 200 µL AL buffer, and re-vortexed until homogeneous. Then 96-100% ethanol was added into the homogenate and was gently mixed until homogeneous.

The incubated suspension was placed into the DNeasy Mini spin column and centrifuged (8000 rpm, 1 minute). The supernatant was transferred into the DNeasy mini spin column, mixed with 500 µL AW1 buffer, and then centrifuged. The supernatant was placed into the DNeasy Mini spin column, mixed with 500 µL AW2 buffer, and centrifuged (14000 rpm, 3 minutes). The supernatant was transferred into a 2-ml microtube and mixed with 200 µL AE buffer. It was then centrifuged (8000 rpm, 1 minute).

PCR methods for catfish

The DNA from the kidneys and liver was amplified using the same primers for the identification of E.ictaluri with the procedures and conditions specified in advance (see Molecular analysis for isolate identification).

LAMP amplification procedures

Primers used in this study were comprised of eip18 gene F3 [TAAGACTCCAGCCCTCGG], B3 [TTCCCTCGCTGGAAGTGG], F1 [GCCCGCAGGAAACCATTGATTTTTTTCCGCCTTACCGCTCTGAT] and B1 [GGCCCCGGAGCGATCATATTTTGCGATAAGTTCGCCTTCTGT] according to the Office International Des Epizooties (2009). Amplification of *E. ictaluri* by the LAMP method using 25 µL reaction consisted of 1X thermopol buffer, 8 U Bst DNA polymerase (New England Biolabs, Beverly, MA), 6 mM $MgSO_4$, 0.8 M betaine, 1.0 mM deoxynucleotide triphosphate, 0.2 µM primers F3 and B3, 1.6 µM primers F1 and B1, 1 µL DNA template. The amplification was performed at 61 °C for 120 minutes and the reaction was stopped at 80 °C for 10 minutes. Then, the results were evaluated by 2% gel agarose electrophoresis stained by SYBR Safe Green.

Statistical analysis

All types of data obtained in this study were analyzed by descriptive statistics to compare between infected and healthy catfish.

RESULTS AND DISCUSSION

Characteristics of E. ictaluri

In this study, the isolates were preserved by freeze-drying. A preliminary test was conducted by culturing the bacteria in TSB and TSA media before the characteristics of the fish were determined. The characteristics were evaluated by PCR and conventional biochemical methods. The incubation of conventional biochemical tests was conducted at 30 °C for 24 hours. Thus, characterization was conducted with 32 characters (Holt et al., 2000). The similarity estimation accounted for 93.75%. Bacterial inoculation from TSB media was extracted using the Qiagen DNeasy blood and tissue kit, and the DNA was amplified by specific *E. ictaluri* primers (Fig. 1a). The confirmation test was performed by sequencing, as shown in Figure 1b. As the Figure shows, the sequence marker obtained from the isolate and then compared with two accessions available in NCBI had 99% query coverage. This means that the amplified product was confirmed to belong to the specific marker of *E. ictaluri* and was ready to be analyzed (Table 1).

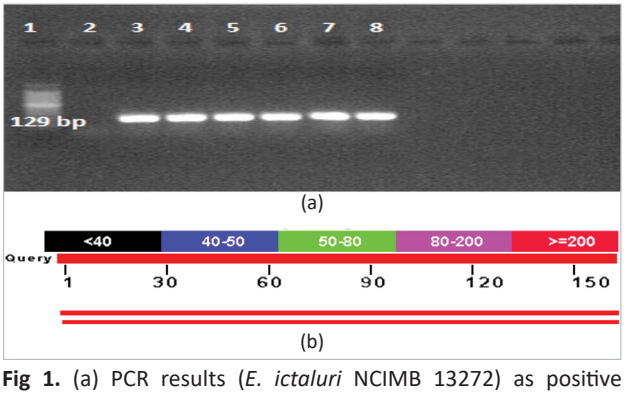

Fig 1. (a) PCR results (*E. ictaluri* NCIMB 13272) as positive control. Lane 1: 100 bp marker; Lane 2: negative control; Lane 3–8: *E. ictaluri* isolate. (b) Color key for alignment scores. Two lines under the query show sequences from two gene references (accession number: CP001600.1 (upper) nd AF110153.1 (lower)).

Table 1. Sequencing results of *E. ictaluri* as a positive control

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
CP001600.1	*Edwardsiella ictaluri* 93-146, complete genome	329	329	99%	3e-87	100%
AF110153.1	Edwardsiella ictaluri phosphoserine transaminase (serC) gene, partial cds; and 5-enolpyruvoylshikimate-3-phosphate synthase (aroA) gene, complete cds	329	329	99%	3e-87	100%

Virulence reversion of E. ictaluri after treatment

After injection, catfish infected with *E. ictaluri* showed clinical symptoms, such as not swimming normally and showing their heads above the water surface (vertical movement). Pathological examination revealed hemorrhages of the skin, fin necrosis, pale hepatic color, swollen kidneys, and a darker spleen (Fig. 2).

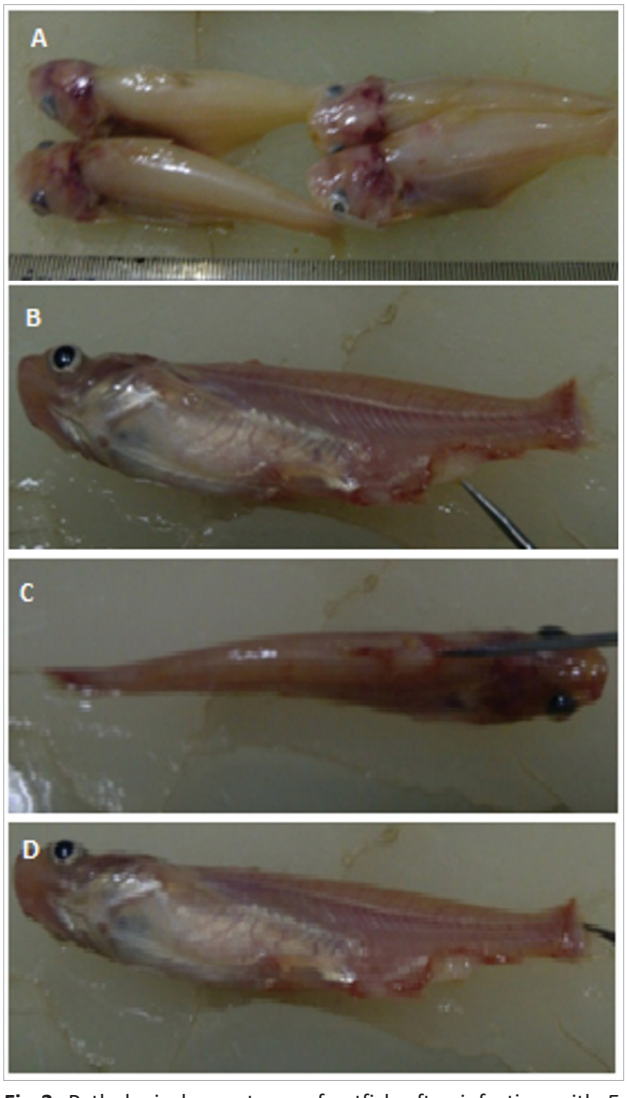

Fig 2. Pathological symptoms of catfish after infection with *E. ictaluri*. Head necrosis (A) and petechial hemorrhages in the fins (B-D)

According to Hawke et al. (1998), pathological lesions in fish suffering from ESC are due to infection with *E. ictaluri*. Furthermore, petechial hemorrhages were found throughout the body, fins, protruding eyes, necrosis, inflammation of the spleen, liver, intestines, pale gills, gut, abdominal swelling, and tissue necrosis in the upper part of the head.

The fish infected with *E. ictaluri* was hanging with the head above and the tail below. The clinical symptoms mentioned above were pathognomonic for *E. ictaluri* infection in the brain. In addition, fish infected with *E. ictaluri* showed clinical symptoms, such as swimming with the head above and the tail below (Hawke et al., 1998).

Biochemical test in fish

Biochemical tests were conducted on each catfish. Kidneys and liver were used for *E. ictaluri* isolation. These organs were cultured on TSA media and incubated at 30 °C for 24 hours. The results of the bacterial culture were purified, and biochemical tests were performed. *E. ictaluri* bacteria were identified by the biochemical test. The characteristics of the biochemical tests had a similar result with 32 characters in the biochemical tests (Holt et al., 2000). The results showed that only three parameters did not match with the ability of *E. ictaluri* in lysine decarboxylase, ornithine and changing acids in sucrose, glycerol. Table 2 shows the results of the NCIMB and isolate tests.

Loop-mediated isothermal amplification test results

Microtubes containing LAMP reactions were amplified at an incubator or water bath with several different temperatures and times. Temperature optimization was performed at 61 °C, 63 °C, 65 °C, and 70 °C, whereas the incubation time was performed at 60, 90, and 120 minutes. The optimization results can be seen in Table 3, which shows all data were identical except for temperatures 61 °C and 63 °C for 90 and 120 minutes of incubation. Table 3 and Figure 3 show the longest duration of amplification and the lowest temperature produced the amplicon much better than the other optimizations.

The different temperatures and incubation times are needed for the optimization results of *E. ictaluri* detection by the LAMP method. According to the Office International Des Epizooties (2009), the incubation temperature must be at 65 °C for 60 minutes.

Table 2. Biochemical tests of *E. ictaluri* between isolate from NCIMB strain and fish

No.	Test	Results		Holt et al.
		NCIMB Isolate	Fish Isolate	(2000)
1	Gram	-	-	-
2	Form	R	R	R
3	Motility	-	-	-
4	Catalase	+	+	+
5	Oxidase	-	-	-
6	Glucose	+	+	+
7	Gas from Glucose	+	+	+
8	OF	F	F	F
9	H_2S	-		-
10	Ornithine	+	-	D
11	Indole	-	-	-
12	Urea	-	-	-
13	Decarboxylase lysine	-	-	+
14	Gelatin	-	-	-
15	Arginine	-	-	-
16	*Simmons citrate*	-	-	-
17	Methyl red	-	-	-
18	Voges Proskauer	-	-	-
19	Nitrate reduction	+	+	+
20	Esculin hydrolysis	-	-	-
21	Phenylalanine	-	-	-
22	Inositol	-	-	-
23	Mannitol	-	-	-
24	Sorbitol	-	-	-
25	Salicin	-	-	-
26	Sucrose	+	+	-
27	Glycerol	-	+	-
28	Melibiose	-	-	-
29	Lactose	-	-	-
30	Arabinose	-	-	-
31	Trehalose	-	-	-
32	Maltose	+	+	+
33	Xylose	-	-	-

Note: R: *Rod*; F: fermentative; +: positive result; -: no result detected

Table 3. The optimization of temperature and time by LAMP amplification

Temperature (°C)	Time (minute)		
	60	90	120
61	-	+	+
63	-	-	+
65	-	-	-
68	-	-	-

Furthermore, the results can be seen at 2% of agarose gel. It is consistent with the results reported by Yeh et al. (2005). Bst DNA polymerase works optimally at 65 °C and lower temperatures. The reaction length affects the target DNA results, i.e. the more extended the reaction, the more increased the DNA target.

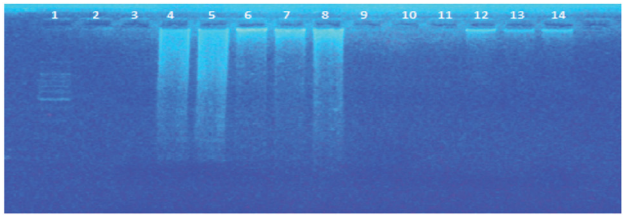

Fig 3. PCR results after optimization of temperature and time by LAMP method. Lane 1: 100 bp ladder; Lane 2: negative control; Lane 3: optimization at 61°C for 60 minutes; Lane 4: optimization at 61°C for 90 minutes; Lane 5: optimization at 61°C for 120 minutes; Lane 6: optimization at 63°C for 60 minutes; Lane 7: optimization at 63°C for 90 minutes; Lane 8: optimization at 63°C for 120 minutes; Lane 9: optimization at 65°C for 60 minutes; Lane 10: optimization at 65°C for 90 minutes; Lane 11: optimization at 65°C for 120 minutes; Lane 12: optimization at 68°C for 60 minutes; Lane 13: optimization at 68°C for 90 minutes; Lane 14: optimization at 68°C for 120 minutes.

Based on the optimization results of temperature and time, the samples were amplified at 61 °C for 120 minutes. Furthermore, it was continued at 80 °C for 10 minutes to stop the LAMP reaction. The amplification results can be seen by electrophoresis. Positive results indicate a change from orange to green (Kono et al., 2004). Test results for samples infected with *E. ictaluri* showed positive results 12 hours after infection (Figs 4a and 4b).

The specificity test was performed to evaluate LAMP primers for *E. ictaluri* detection. *E. ictaluri*, *Edwardsiella tarda*, *Aeromonas salmonicida*, and *Aeromonas hydrophylla* were used for sensitivity tests. The results show that the LAMP method using the eip18 gene primers was very specific in detecting *E. ictaluri* (Fig. 5). The eip18 gene primers specifically detect *E. ictaluri* presence and infection (Yeh et al., 2005).

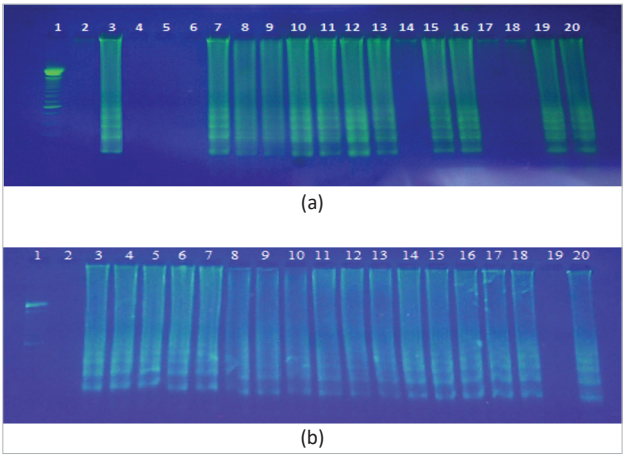

Fig 4. (a) PCR results in a fish sample by LAMP. Lane 1: 100 bp ladder; Lane 2, 14, and 17: negative control; Lane 3: positive control; Lane 4: 6 hours post infection (p.i); Lane 5: 6 hours p.i; Lane 6: negative control 6 hours p.i; Lane 7: 12 hours p.i; Lane 8–9: 12 hours p.i; Lane 10–13: 18 hours p.i; Lane 15: 24-hour p.i; Lane 16: 21 hours p.i; Lane 18: negative control sample; Lane 19–20: 30 hours p.i. (b) Continued LAMP results in fish samples. Lane 1: 100 bp ladder; Lane 2 and 19: negative control; Lane 3: positive control; Lane 4–5: 36 hours p.i; Lane 6–7: sample 42 hours p.i; Lane 8–9: sample 48 hours p.i; Lane 10: sample 48 hours p.i; Lane 11–13: sample 54 hours p.i; Lane 14–16: H + 3 p.i; Lane 17–18: sample H + 5 p.i; Lane 20: H + 7 p.i.

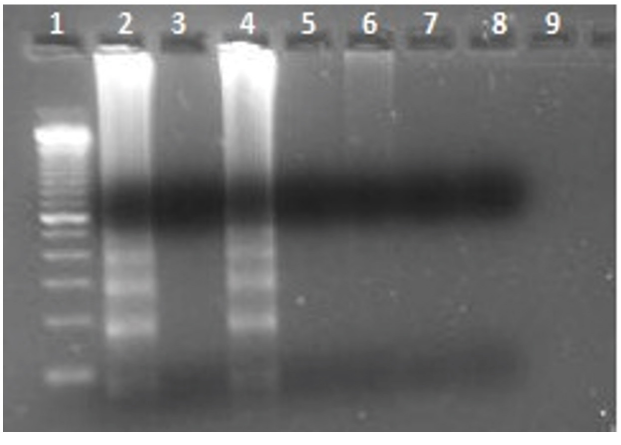

Fig 5. PCR result of specificity test using LAMP method. Lane 1: 100 bp ladder; Lane 2: positive control; Lane 3: negative control; Lane 4: *E. ictaluri*; Lane 5: *Aeromonas hydrophylla*; Lane 6: *E. tarda*; Lane 7: *A. salmonicida*.

PCR for catfish infected with E. ictaluri

The kidney of fish infected with *E. ictaluri* was taken, and PCR was performed with specific primers for *E. ictaluri* (Iwanowicz et al., 2009). According to Fig. 6, it can be seen that the infection was detected in 24 hours after injection.

Comparison between LAMP and PCR

The sensitivity test was performed to determine the primary sensitivity of LAMP. It was performed to detect *E. ictaluri*.

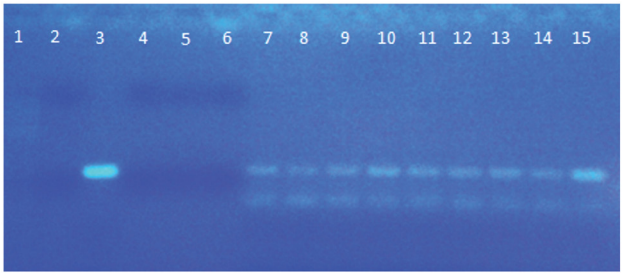

Fig 6. PCR results of kidney infected with *E. ictaluri* in catfish. Lane 1: 100 bp marker; Lane 2: negative control; Lane 3: positive control; Lane 4: 6 hours p.i; Lane 5: 12 hours p.i; Lane 6: 18 hours p.i; Lane 7: 24 hours p.i; Lane 8: 30 hours p.i; Lane 9: 36 hours p.i; Lane 10: 42 hours p.i; Lane 11: 48 hours p.i; Lane 12: 54 hours p.i; Lane 13: day 3 p.i; Lane 14: day 5 p.i; Lane 15: day 7 p.i.

PCR test showed the specific primers for *E. ictaluri* could only detect up to ten thousand times as much as the actual concentration or 103 CFU/ml, whereas the LAMP test could detect all concentrations (Fig. 7). In addition, the LAMP test produced many more bands of different sizes than the PCR method, which detected two bands in each upper lane These results revealed that the LAMP method was more sensitive than the PCR method. It was due to the ability to detect in 12 hours after infection. The LAMP test has a high sensitivity with four specific primers and recognizes six targets of DNA regions (Yeh et al., 2005). Sample evaluation with the PCR, nested PCR, and LAMP methods has a positive accuracy of 60%, 70%, and 70%, respectively (Tsai et al., 2009).

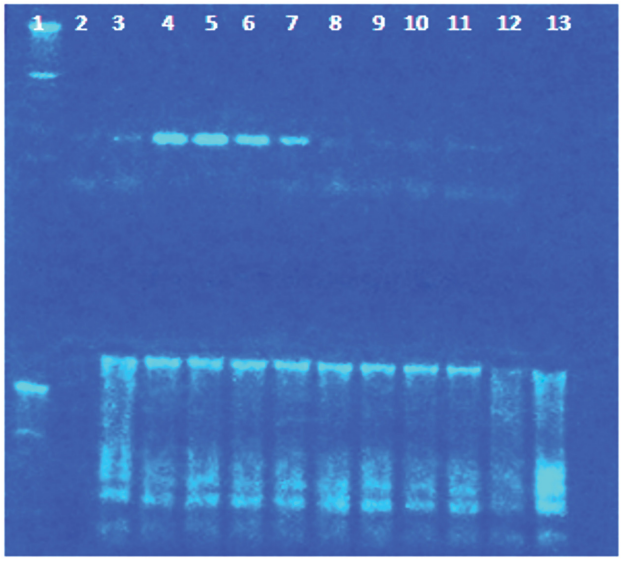

Fig 7. Results of sensitivity tests between PCR (above) and LAMP (below). Lane 1: 100 bp ladder; Lane 2: negative control; Lane 3: positive control; isolate with serial dilution concentrations: Lane 4: 10^{-1}; Lane 5: 10^{-2}; Lane 6: 10^{-3}; Lane 7: 10^{-4}; Lane 8: 10^{-5}; Lane 9: 10^{-6}; Lane 10: 10^{-7}; Lane 11: 10^{-8}; Lane 12: 10^{-9}; Lane 13: positive control (LAMP lane).

CONCLUSIONS

This study shows that the LAMP method has higher sensitivity and specificity than PCR to detect infection with *E. ictaluri* in catfish. This method can be an effective and robust way to help the detection of fish disease caused by bacterial infection.

ACKNOWLEDGMENTS

This work was supported by the fish quarantine standard test center for quality control and safety of fishery products.

PRIMJENA IZOTERMNE AMPLIFIKACIJE POSREDOVANE PETLJOM ZA DETEKCIJU *Edwardsiella ictaluri* U INDONEZIJSKOJ CRIJEVNOJ SEPTIKEMIJI SOMA

SAŽETAK

Metoda izotermalne amplifikacije posredovane petljom (LAMP) korisna je za brzo otkrivanje infekcije *Edwardsiella ictaluridom*, a posebno crijevne septikemije soma (ESC). Ova studija ima za cilj istražiti LAMP metodu za otkrivanje *E. ictaluri* kod ESC bolesti. Ova je eksperimentalna studija koristila ukupno 55 somova, od koji je 5 negativnih kontrola, a u 50 somova je intraperitonealno ubrizgano 0,1 mL *E. ictaluri* u koncentraciji od 105 CFU/mL. Bubrežni organi triju riba nasumično su odabrani 6, 12, 18, 24, 30, 36, 42, 48 i 54 sati nakon infekcije s *E. ictaluri*. Osim toga, uzorci su također prikupljeni 3., 5. i 7. dana. Bakterijska analiza određena je konvencionalnom biokemijom (test roda i vrste), ekstrakcijom cijelog genoma soma i LAMP pojačanjem. Sve vrste podataka dobivene u ovoj studiji analizirane su deskriptivnom statistikom kako bi se usporedili zaraženi i zdravi somovi. Som je bio zaražen *E. ictaluri* nakon 12 sati od infekcije prema LAMP postupku s kojim su pronađena krvarenja po cijelom tijelu, perajama, izbočene oči, nekrozu, upalu slezene, jetre, crijeva, blijede škrge, crijeva, oticanje abdomena i nekrozu tkiva u gornjem dijelu glave. Može se zaključiti da je LAMP metoda učinkovitija u otkrivanju infekcije *E. ictaluri* među somovima od PCR metode.

Ključne riječi: bakterije, somovi, dijagnostičke metode, bolesti riba, molekularna dijagnostika

REFERENCES

Gustiano, R., Prakoso, V. A., Radona, D., Dewi, R. R. S. P. S., Saputra, A., Nurhidayat. (2021): A sustainable aquaculture model in Indonesia: multibiotechnical approach in *Clarias* farming. In The 3rd International Conference on Fisheries and Marine Sciences (IOP Conf. Series: Earth and Environmental Science, 718, 012039.

Halver, J. H., Hardy, R. W. (2002): Fish nutrition (3rd ed.). Massachusetts: Academic Press.

Hawke, J. P., Durborow, R. M., Thune, R. L., Camus, A. C. (1998): ESC-enteric septicemia of catfish (Vol. 477). Mississippi: Southern Regional Aquaculture Center.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., Williams, S. T. (2000): Bergey's manual of determinative bacteriology (9th ed.). Philadelphia: Lippincott, Williams & Wilkins.

Iwanowicz, L. R., Blazer, V. S., McCormick, S. D., VanVeld, P. A., Ottinger, C. A. (2009): Aroclor 1248 exposure leads to immunomodulation, decreased disease resistance and endocrine disruption in the brown bullhead, Ameiurus nebulosus. Aquatic Toxicology, 93(1), 70-82.

Kono, T., Savan, R., Sakai, M., Itami, T. (2004): Detection of white spot syndrome virus in shrimp by loop-mediated isothermal amplification. Journal of Virological Methods, 115(1), 59-65.

Muchtadi, T. R., Sugiono. (1992): Food science. Ministry of Education and Culture. Bogor: Bogor Agriculture Institute.

Office International des Epizooties. (2009): Manual of diagnostic test for aquatic animals, enteric septicaemia of catfish (*Edwardsiella ictaluri*). Paris: Office International Des Epizooties.

Savan, R., Igarashi, A., Matsuoka, S., Sakai, M. (2004): Sensitive and rapid detection of edwardsiellosis in fish by a loop-mediated isothermal amplification method. Applied and Environmental Microbiology, 70(1), 621-624.

Tsai, S. M., Chan, K. W., Hsu, W. L., Chang, T. J., Wong, M. L., Wang, C. Y. (2009): Development of a loop-mediated isothermal amplification for rapid detection of orf virus. Journal of Virological Methods, 157(2), 200-204.

Wise, A. L., LaFrentz, B. R., Kelly, A. M., Khoo, L. H., Xu, T., Liles, M. R., Bruce, T. J. (2021): A Review of Bacterial Co-Infections in Farmed Catfish: Components, Diagnostics, and Treatment Directions. Animals, 11(11), 3240.

Yeh, H. Y., Shoemaker, C. A., Klesius, P. H. (2005): Evaluation of a loop-mediated isothermal amplification method for rapid detection of channel catfish (*Ictalurus punctatus*) important bacterial pathogen *Edwardsiella ictaluri*. Journal of Microbiological Methods, 63(1), 36-44.