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Multiplex Analysis of Animal Species Origins in Feedstuffs and Foodstuffs for the Prevention, Control and Eradication of Transmissible Spongiform Encephalopathies (TSEs) by PCR

Muhammad SAFDAR
Gaziantep University

Mehmet OZASLAN
Gaziantep University

Abstract: Recently, highly strict regulations for the prevention, control, and elimination of transmissible spongiform encephalopathies (TSEs) were put in place by the European Parliament and the Council of the European Union. Furthermore, because of health concerns, there is a greater demand for knowledge about the composition of feeds and foods, particularly pet food and ruminant feed, so determining the species of origin is critical. Therefore, there is a dire need for an advanced technique that should be rapid, specific, and inexpensive. The purpose of this study was to develop an M-PCR (multiplex PCR) assay that could identify TSEs causing origin species concurrently in food and feed products while employing fluorescent dyes that were less expensive than doubly labelled probes. The development and optimization of M-PCR allowed for the analysis of the origin of animal species' DNAs in complicated feed and food matrices. The primers were designed using specific segments of DNA sequences of the targeted species (bovine: 271bp, ovine: 119bp, caprine: 224bp). The optimized M-PCR assay may be a useful tool for confirming the species origin of feedstuffs and commodities subjected to denaturing technologies, according to the assay's results.

Keywords: PCR, Multiplex analysis, species origin identification, Transmissible Spongiform Encephalopathies

Introduction

The neurological condition called as bovine spongiform encephalopathy (BSE), also referred to as mad cow disease, is lethal and untreated in cows. Atypical conduct, difficulty walking, and weight loss are major symptoms. It is a subtype of scrapie in sheep and Creutzfeldt-Jakob disease in humans (Nash et al., 2009; Singeltary, 2016; Singh et al., 2019). The European Food Safety Authority and Regulation (EU) No 1372/2021 adopted measures to limit the presence of processed animal proteins (PAPs) directly or indirectly in ruminant feed in order to reduce the risks of BSE to humans and animals (Focker et al., 2022; Lourenço et al., 2022).

For the qualitative examination of animal species in feed and food items, some researchers have used traditional gel electrophoresis-based PCR-detection (Safdar, 2013; Safdar & Junejo, 2015, 2016). As an alternative, multiplex PCR is a quick, affordable, and simple method to use DNA for commercial analysis and feed surveillance (Safdar & Junejo, 2015; Safdar et al., 2014a; Safdar et al., 2014b). Consequently, a cutting-edge technology that is quick, precise, and affordable is desperately needed (Safdar & Junejo, 2016). This study's objective was to design a M-PCR (multiplex PCR) assay that could simultaneously identify the species that cause TSEs in food and feed products while using fluorescent dyes that were less expensive than doubly labelled probes.

Methods

Samples

Reference samples were prepared at the molecular laboratory to optimise simplex and multiplex PCR assays under control conditions. All the requirements were applied as they are used in the commercial samples. 150 samples were collected from the different commercial markets to evaluate our optimised PCR. Samples were collected from January 2022 to February 2022. The collected samples were then immediately transferred to the Molecular lab and stored at +4°C for further investigation.

DNA Extraction

The DNA extraction from both laboratory-prepared and commercial samples was done according to the Qiagen tissue kit (Germany) instructions and the DNA was quantified by the NanoDro 2000 Spectrophotometer (USA). Further studies were conducted on the quality of DNA.

PCR

For the simplex and triplex PCR amplification, a final volume of 25 l, 10 pmol of each species' ovine, bovine, and fish primers, and 90 ng of DNA template were used. Amplification was carried out in a Thermocycler BioRad under the following cycling conditions: The 35 cycles were as follows: 94 °C for 30 seconds, 60 °C for 1 minute, 72 °C for 1 minute, and a final 5 minute extension at 72 °C. The initial heat denaturation phase was carried out at 94°C for 10 min. The PCR-amplified products were stained with ethidium bromide (10 ng/ml) for 20 minutes after electrophoresis on a 2 percent agarose gel (Helicon, USA) in 0.5X TBE buffer (Trisbase, Boric acid, NaEDTA) for 50 minutes at 100 V. A camera was used to capture a picture of the agarose gel while it was being seen under UV light (Vilber Lourmat BP 66, France) (Rainbow S6X11DC4P, Traverse City, MI, USA).

Results and Discussion

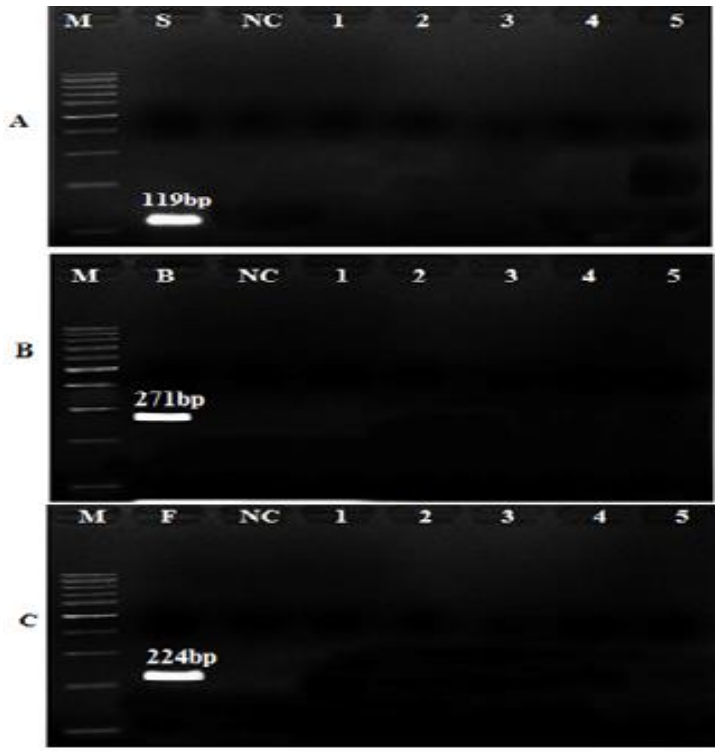

Figure 1. Specificity of primers with respective DNA. M:100bp ladder, S: sheep, B: cow, F: goat, NC: negative control, 1: buffalo, 2: camel, 3: pork, 4: horse, 5: chicken

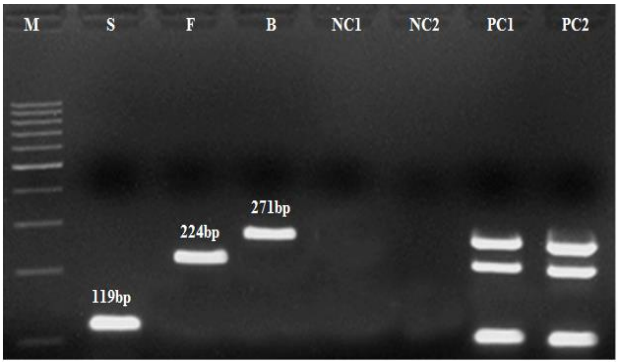

Figure 2. Optimization of triplex PCR M:100bp ladder, S: sheep, B: cow, F: Goat, NC: negative control, PC1: positive control

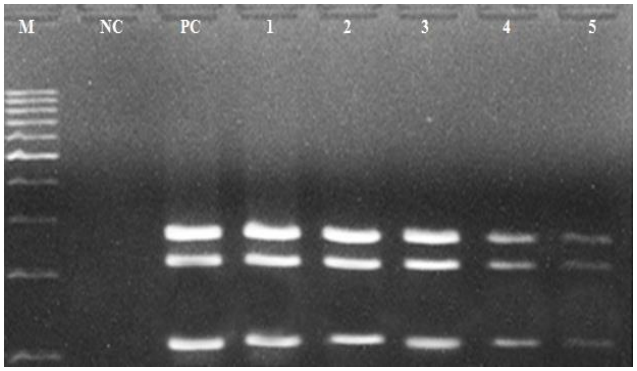

Figure 3. Sensitivity of triplex PCR: M:100bp ladder, NC: negative control, PC: positive control, 1; 5%, 2; 5: 3; 1%, 4; 0.1%, 5; 0.01%

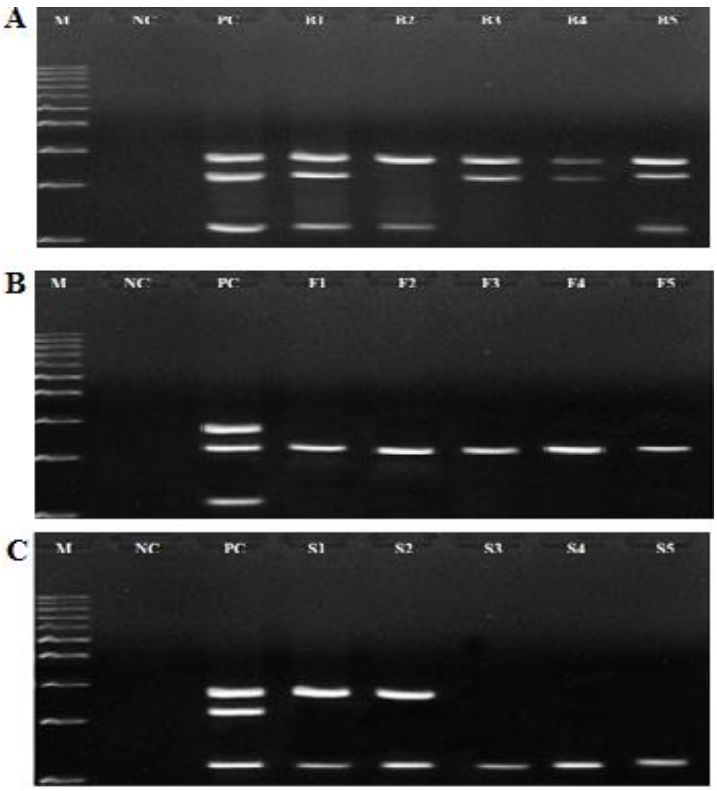

Figure 4. Application of Triplex PCR. M:100bp ladder, NC: negative control, PC: positive control, B1-B5; cow feeds, F1-F5: goat feeds, S1-S5: sheep feeds

The use of the developed method on industrial feed samples is shown in Figure 1-4, which accurately indicates the species present in the commercial feed samples that were supplied. The method revealed that laboratory

prepared samples of cattle, sheep, and goat origin contained exactly the same things that were labelled by us. Similarly, the commercial samples were investigated and found to have the contrary results (Figure.4). Laboratory samples indicated that all samples were appropriately confirmed at 100%. Comparing our results to those in the literature showed that they were about the same or better in terms of specificity, sensitivity, and cost (Denyingyhot et al., 2022; Edwards & Gibbs, 1994; Galal-Khallaf, 2021; Lourenço et al., 2022; Roig et al., 2022; Sangthong et al., 2021; Yanaso et al., 2021; Zhao et al., 2021).

Conclusion

Based upon our results, it has been concluded that this multiplex PCR could detect sources of TSEs related to animal species in foodstuffs and feedstuffs. The assays developed in this study have potential as molecular tools that can be used for rapid and routine detection of animal DNA in feedstuffs simultaneously. It could be used by government institutes to find and identify all of these species in food products at the same time.

Recommendations

This article will lead the scientists to focus on rapid, specific and economical multiplex PCR for identification of TSEs to control and eradicate this.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

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Author Information

Muhammad SAFDAR
Department of Biology, Division of Molecular Biology and Genetics, Gaziantep University, Gaziantep 27310, Turkey
Email: drmuhammadsafdar8@gmail.com

Mehmet OZASLAN
Department of Biology, Gaziantep University
Gaziantep 27310, Turkey

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