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Simplex Immunodotblot for detecting dog meat contaminants in foodstuffs

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Abstract

This study is an initial effort to develop a prototype kit based on the simplex immunodot blot method to detect dog meat contamination in food products. The simplex immunodotblot is an immunoassay technique that identifies specific proteins or antigens through antibody-antigen interactions, targeting a single contaminant in one reaction. The antigen utilized was dog meat extract (DME) prepared in the laboratory, while the primary antibodies (Ab) against DME were produced in-house using laying hens. Test samples were applied in varying concentrations (5, 10, and 20 µg per spot) onto comb-shaped polyester plastic sheets. A commercial secondary antibody against IgY, labeled with horseradish peroxidase (HRP), served as the detector. The reaction was visually inspected; in cases where dog meat was present, a blue or black spot appeared due to the reaction between the antigen, antibody, enzyme, and substrate. The study demonstrated the successful development of the simplex immunodotblot, which was able to detect dog meat visually at concentrations as low as 5 µg in a laboratory setting. However, further research is required, particularly to adapt the prototype kit for halal food authentication purposes.

Keywords: Antibody; Beef; Dog meat; Halal; Immunodotblot; Meat extract

1. Introduction

Food safety, particularly concerning livestock-derived products like fresh and processed meats, has long been a critical issue. Consumers not only prioritize the taste and appearance of their food but also demand assurance regarding its safety and nutritional quality, especially for livestock-based products (1). Improper handling of raw materials and processed livestock foods can lead to food poisoning and heighten the risk of meat-borne epidemics (2).

Beyond food safety, religious considerations also play a significant role. For Muslims, consuming non-halal meat is prohibited. Species like dogs, pigs, and rats are classified as non-halal and are also known vectors of infectious diseases, particularly when sourced from wild animals or those poorly raised (3) ,(4), (5). Inadequate meat processing further exacerbates health risks for consumers.

In countries like Indonesia, cases of meat adulteration persist, where pork or dog meat is illicitly mixed with beef to produce items like meatballs, primarily due to the lower cost of these meats compared to beef (6). Similar cases have been reported in Egypt (7). These economic-driven adulterations have spurred research over the past decade into detection methods for such practices. Numerous studies have focused on developing protein and nucleotide-based testing kits to identify meat adulteration (7), (8), (9), (6).

This paper presents findings from our preliminary study on creating a practical testing kit—a simplex immunodot blot—for detecting dog meat contamination in food products.

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2. Material and methods

2.1. Material preparation

This study utilized an antigen derived from dog meat purchased from a specialized seller in Mataram City, West Nusa Tenggara, Indonesia. Dog meat extract (DME) was prepared in the laboratory by manually grinding the meat with a porcelain mortar, following a modified method based on Nuhaerani et al. (10) and Kinenda et al. (11). For every gram of meat, 3 ml of physiological saline solution was added. The mixture was then centrifuged at 4500 rpm at 4°C for 30 minutes. The resulting pellet was resuspended in physiological saline solution and centrifuged again under the same conditions. The resulting supernatant, termed the meat extract, was aliquoted and stored at -20°C for future use. Beef, chicken, and mutton extracts were prepared using the same process, with precautions taken to prevent cross-contamination between species. The protein concentration of each meat extract was measured using a spectrophotometer at OD280.

The primary antibody (Ab) against the dog meat extract was a polyclonal IgY antibody, produced in-house using laying hens, as described by Depamede et al. (12). While a secondary antibody (Goat Anti-Chicken IgY HRP) as detector was purchased from Invitrogen (USA).

2.2. Testing process

The process was carried out based on Kinenda et al. (11) with slight modification. Samples were applied at 5 µl per dot on comb-shaped polyester plastic sheets (referred to as "combs"). Each spot represented a specific dilution, i.e. concentrations of 20, 10 and 5 µl per spot. The samples were dried at 37°C for 60 min, then blocked with 4% skim milk for 60 min at the same temperature. The combs were washed three times with PBS containing 0.05% Tween 20.

Next, the spots were incubated with a primary antibody (Chicken anti-Dog IgY) at 37°C for 60 minutes, followed by washing. A secondary antibody (Goat Anti-Chicken IgY HRP) was applied under the same conditions. After a final wash, the spots were treated with TMB, an HRP substrate. The reaction was stopped by adding distilled water.

2.3. Interpretation of results and data analysis

The presence of dog meat in the sample was indicated by the formation of blue or dark-blue spots, resulting from the antigen-antibody-enzyme-substrate interaction. These results were visually observed and documented by photographing. All experiments were conducted or repeated at least twice

3. Results and discussion

The primary goal of this research is to develop a practical method for detecting dog meat contamination. While dog meat consumption is acceptable in certain countries or regions, it is prohibited or frowned upon in others, often due to religious restrictions (13), (6). However, because dog meat is relatively inexpensive, unscrupulous traders may use it to adulterate meat from other species, such as beef, goat, or lamb. Therefore, effective and practical detection methods are essential. One such method is the immunodot blot technique. Preliminary findings indicate that the immunodot blot method, applied to comb-shaped polyester plastic sheets, has the potential for further development (Figure 1).

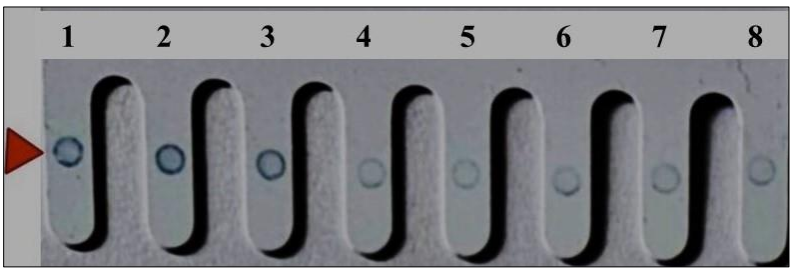

Figure 1 Representation of the results of the development trial of dog meat contamination detection using immunodotblot on comb-shaped polyester plastic sheets. The red arrowhead sequentially indicates: 1-3 represent dog meat extracts at concentrations of 20 µg, 10 µg, and 5 µg, respectively; followed by 4-8 representing chicken meat (10 µg), rat meat (10 µg), pork (10 µg), beef (10 µg), and mutton (10 µg)

Figure 1 presents the results of the immunodot blot test, demonstrating that the prototype developed in this study is highly specific to detecting dog meat. The blackish spots corresponding to dog meat are noticeably darker than those produced by chicken, rat meat, pork, beef, or goat meat. This suggests that the method holds promise for further development as a simple tool to detect food fraud involving dog meat.

While both immunoblot and ELISA are protein-based assays, immunoblot is generally considered less sensitive, particularly when compared to DNA-based methods like PCR and its derivatives (7), (8). However, the immunoblot method developed here shows potential for practical field applications. The prototype effectively detects dog meat visually at concentrations as low as 5 µg, while spots from other species at 10 µg appear significantly fainter.

This study does have limitations. The data is currently insufficient, and no direct comparisons have been made with other methods such as ELISA or PCR. Additionally, the design employs a sandwich immunoassay format, requiring the analyte to be dripped onto a comb, whereas an ideal design would allow direct application of the analyte to be tested. These shortcomings highlight areas for improvement in future research.

Figure 1, which represents the results of the immunodotblot test, visually shows that the prototype developed in this study is specific to dog meat, as the blackish spot color is more obvious than the spots produced by chicken, rat meat, pork, beef, or goat meat. These results indicate that this method has the potential to be further developed to more simply detect food fraud that uses raw materials derived from dog meat.

Compared to ELISA, although both are protein-based assays, immunodotblot is recognized to be less sensitive than ELISA; even more so when compared to DNA-based assays such as PCR and its derivatives (7), (8). However, in terms of practical use in the field, the immunodotblot developed in this study is promising. Visually, the developed kit able to detect the presence of dog meat clearly up to a concentration of about 5 µg compared.

The weakness of this study was that the data were still lacking, no direct comparative study was conducted with other methods such as ELISA and PCR. In addition, the design used was a sandwich immunoassay format where the target analyte was dripped on a comb. Ideally, the user should only have to apply directly to the analyte to be tested. These weaknesses still need to be addressed in future studies.

4. Conclusion

In conclusion, this preliminary study shows that a prototype simplex immunodot blot kit has been successfully developed. The prototype was capable of detecting dog meat contamination at levels as low as 5 µg. However, further study is still needed to address certain limitations and improve the effectiveness and feasibility of the kit.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Some of the information in this paper was presented at the National Seminar on Science and Technology, the Institute for Research and Community Engagement, University of Mataram, on November 07, 2024, in Mataram, Indonesia.

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