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Species identification of meat by monoclonal antibody indirect enzyme linked immunosorbent assay

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In India, identification of the species origin of meat with respect to cattle and buffalo acquires immense importance in forensic medicine since cattle slaughter is prohibited in majority of the states, export of cattle meat (beef) is banned while buffalo meat is permitted. The paper describes the maiden approach on identification of phylogenically closely related species that of buffalo and cattle by indirect-ELISA using monoclonal antibody (mAb).

The buffalo IgG_2 specific mAb (binding specificity for heavy chains of buffalo IgG_2 belonging to IgG_1 subclass) secreted by the hybridoma clone I1AF11.1 was raised and used as detector antibody. To obtain the mAb, the clone prepared and characterized by Subash (1991) was subcultured and propagated first *in vitro* as a static monolayer culture and then in suspension culture to obtain adequate number of cells for growth *in vivo* as peritoneal tumer in BALB/c mice. The mice were primed with pristane (2, 6, 10, 14-tetramethyl pentadecane) by injecting 0.5 ml intra peritoneally (i/p) about 20 days prior to injection of cells. Each one of the primed mice was injected i/p with 1 ml of suspension culture containing 2×10^6 live cells /ml. On development of ascitic tumor (12 to 21days of injecting the hybridoma cells) the ascitic fluid from the above mice was collected, clarified and stored frozen at –20°C till use.

Press fluid extract (PFE) muscle protein antigens from known beef, buffalo meat, chevon (goat meat) and mutton (sheep meat) samples were prepared by wrapping approximately 100-150 g of respective meat samples in muslin cloth and squeezed hard. The fluid obtained was clarified and supernatant was used as PFE muscle protein antigens. The PFE antigens from these 4 species independantly made were tested by the procedure of solid phase indirect-ELISA (Zade 1995). In the laboratory the PFE antigens of buffalo meat and beef were mixed with each other and separately with PFE antigens of mutton and chevon to obtain 0.5 to 50% adulteration. The adulterated mixtures were then tested by indirect-ELISA. The results were analyzed to study the ability of the test to detect minimum possible level of adulterant buffalo meat in beef, mutton and chevon.

All the buffalo test antigens studied under the assay conditions used, produced colour reaction though the highest dilution giving positive reaction varied with individual samples. With quite a few samples the highest dilution studied (1:1 280) gave positive results while all the samples up to 1:320 dilutions revealed colour development. None of the cattle, goat and sheep antigens tested reacted positively under the similar conditions of testing though background in 1:10 dilution was observed in some samples.

The developed indirect-ELISA could detect adulterant buffalo meat in mixtures of other meats (beef, mutton, chevon) at 0.5% level (lowest tested adulteration level) indicating the sensitivity of the test is in the range of results reported by

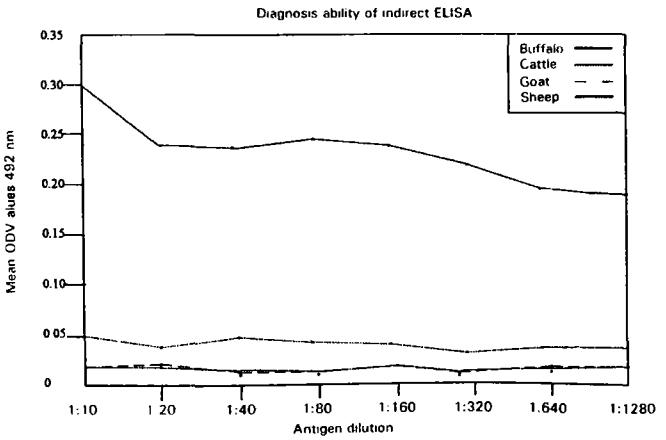

Fig. 1. Diagnosis by indirect ELISA.

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Garcia *et al.* (1994) for horse meat and Morales *et al.* (1994) for pig meat using mAb in indirect-ELISA.

In all 28 buffalo PFE antigens were tested along with equal number of cattle PFE antigens processed and stored identically with 5 each of sheep and goat PFE antigens. All the 28 buffalo samples were identified as buffalo and all 38 (comprising 28 cattle and 5 each of sheep and goat) as non-buffalo indicating cent per cent specificity and sensitivity thus the accurate diagnosis ability of the developed test. The procedure, however, needs to be simplified especially in relation to the dilutions to be used in the test. Most of the buffalo samples revealed perceptive colour development up to dilutions of 1:320 and a few up to 1:1 280. Intensity of colour development receded with dilutions of antigens though the highest dilutions giving positive reaction varied from sample to sample. The result indicates that adequate amount of blood residues were present in all the buffalo meat samples since the mAb used as diagnostic antibody in the present investigation was against buffalo specific IgG_2 molecule. The diagnosis ability of the ELISA procedure developed using this mAb depended upon residual blood proteins in the meat samples known to vary markedly from animal to animal within the same species. Based on the results obtained in the study it may be recommended that the antigen dilutions of 1:20, 1:40 and 1:80 may be best suited for the assay.

SUMMARY

The indirect enzyme linked immunosorbant assay (ELISA) using buffalo specific I_gG_2 monoclonal antibody secreted by hybridoma clone I_1 AF 11.1, was evaluated for its application in identification of raw buffalo meat. The test could detect as low as 0.5% of adulterant buffalo beef in mixture of beef, cheron and mutton, making it many more times sensitive than now available serological/ biochemical tests for identification of buffalo meat.

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