

## Diagnosis of Egyptian Bovine Meat Borne Zoonosis

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**Abstract:** Food borne zoonoses have a major health impact in both industrialized and developing countries. Meat might be infected with some bacterial and parasitic agents; that could be threatening on human health. One hundred and eight meat samples (20 buffaloes and 88 cows) were collected from different Cairo abattoirs and examined parasitologically and bacteriologically. Results showed that 16.67% and 34.26% of the examined meat samples were infected with bacteria and parasites, respectively. The bacterial isolates were non typhoid *Salmonella* (50%), *E. coli* O157:H7 and *Mycobacterium bovis* (1.11%). Three out of the *E. coli* isolates (16.67%) were identified as *E. coli* O157:H7. The liberated parasites were *Cysticercus bovis* (51.35%) and *Toxoplasma gondii* (48.65%). ELISA results showed that seroprevalence of toxoplasmosis was 47, 22.7 and 38.42% in human, cows and buffaloes, respectively. The immunoreactive profiles of *C. bovis* (167.82, 137.32, 88.839, 66.859, 59.851, 54.660 and 48.480 KDa) and *T. gondii* local tachyzoite (158, 111, 102, 86, 55 and 33 KDa) antigens probed with rabbit hyper immune serum showed one immunoreactive band at 55 KDa. While those of *E. coli* (182.01, 144.90, 72.558, 60.324, 28.312 and 18.392 KDa) and non typhoid *Salmonella* (91.967, 60.955 and 20.031 KDa) antigens displayed one common immunoreactive band at 60 KDa. It can be concluded that although immunoblotting help in identification of strains and detection of common cross reactive epitopes between different pathogens, there still exist many challenges and opportunities to improve the current technology of food pathogen detection.

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### 1. Introduction:

Food borne diseases continue to be a major public health problem and constitute an important cause of morbidity and mortality in both developed and developing countries. The annual incidence of some 1.5 billion episodes of diarrhea in children less than 5 years of age, and the more than 3 million resultant deaths are an indication of the magnitude of the problem (NIH, 2006). Most of these diseases are infections caused by a variety of bacteria, viruses and parasites (Smith, 2003).

Meat is an important source of protein and a valuable commodity in resource poor communities. In many developing countries, there is lack of appropriate slaughtering facilities, unsatisfactory slaughtering techniques and slaughtering places are frequently contaminated and may not be protected against dogs, rodents and insects. Meat products coming from such conditions are often deteriorated due to bacterial infection or contaminated, which may cause food poisoning or diseases in consumers. Moreover, in many developing countries, regulations concerning meat inspection and/or control are inadequate or non-existent allowing consumers to be exposed to pathogens including zoonotic parasites (Joshi et al, 2003).

In many countries of the world, bacterial food borne zoonotic infections are the most common cause of human intestinal disease (Thorns, 2000). Salmonellosis is the most common food borne bacterial disease in the world. *Salmonella* is a significant pathogen for food-producing animals and these animals are the primary source of salmonellosis. It is estimated that herd prevalence varies between 0% and 90%, depending on the animal species and region (Plym and Wierup, 2006). Shiga toxin-producing *E. coli* (O157: H7) usually do not cause disease in animals but may cause watery diarrhea, hemorrhagic colitis, and/or hemolytic uremic syndrome in humans. Cattle and other ruminants are the most important reservoir of zoonotic *E. coli* O157, which are transmitted to humans through the ingestion of foods or water contaminated with animal feces (Fairbrother and Nadeau, 2006). *Mycobacterium bovis* infections are of major importance in many developed and developing countries. Transmission of *M. bovis* can occur between animals, from animals to humans and vice versa and rarely, between humans. As with *M. tuberculosis*, transmission is most common by the aerosol route and also through the ingestion of milk and meat from infected animals. Infection acquired

through ingesting *M. bovis* is more likely to result in non-pulmonary forms of disease (Oloya et al., 2007).

Parasitic food borne diseases remain a major public health problem affecting hundred millions of people and animals, particularly in tropical developing countries (Tagboto and Townson, 2001). Bovine cysticercosis is a zoonosis caused by the larval stage (cysticercus) of the human tapeworm *Taenia saginata*. Humans get infected by eating raw or undercooked meat containing viable cysticerci. They penetrate the intestinal wall and invade subcutaneous tissue, brain, eye, muscle, heart, liver, lung, and peritoneum. Bovine cysticercosis occurs world-wide, but is at particularly high prevalence in Africa (Eddi et al., 2003). Meat borne toxoplasmosis in humans may result from ingestion of tissue cysts contained in meat of many different animals (Tenter, 2009). Up to 50% of *T. gondii* infections are transmitted by ingesting undercooked meat, making toxoplasmosis one of the most clinically significant food borne diseases in pregnant women (Ogunmodede et al., 2005).

The types of food borne diseases are constantly changing. A century ago; typhoid and Cholera were common food borne diseases. Improvement in food safety as pasteurization of milk, safe canning and disinfection of water has conquered those diseases. Today new food borne diseases took their place as *Salmonella Enteritidis*, *Campylobacter* and *E. coli* O157: H7. In future, other diseases; whose origin is currently unknown may turn out to be related to food born infections. So, in this investigation we shall try to diagnose bovine bacterial and parasitic meat borne infections common in Egypt.

## 2. Material and Methods

### 1. Sample collection:

**a. Meat samples:** One hundred and eight cattle meat samples (20 buffaloes and 88 cows) were collected from different Cairo abattoirs and subjected for bacteriological and parasitological examination.

**b. Blood samples:** Two hundreds, 20 and 88 blood samples were collected from the contact persons, the investigated buffaloes and cows, respectively and used for the determination of the prevalence of *T. gondii* infection.

### 2. Bacteriological meat examination:

Samples were taken from the infected lymph nodes, lungs, liver, spleen and kidneys of the infected animals (buffaloes and cows) and inoculated on different bacteriological media {nutrient agar, MacConkey agar, blood agar, *Salmonella Shigella* (SS) agar, Lowenstein-Jensen}. Samples were also

enriched with tryptone soya broth containing novobiocin and sub cultured on sorbitol MacConkey agar for isolation of *E. coli* O157. The inoculated plates were incubated at 37°C for 48hr but cultures on Lowenstein-Jensen were incubated up to 8 weeks. Suspected colonies appearing on the different media were identified according to Holt et al. (1994) and Roberts et al. (1991) for *Mycobacteria*. The recovered *Salmonella* isolates were identified serologically using the diagnostic polyvalent and monovalent antisera according to Kauffmann (1972). *E. coli* O 157 suspected colonies were tested by the *E. coli* O157:H7 agglutination latex test (Oxoid ).

### 3. Parasitological meat examination:

**a. Cysticercus bovis cysts** were collected from liver, heart and masseter muscles of infected cattle.

**b. *T. gondii* local isolate** was isolated according to procedures described by Dubey and Beattie (1988) after many trials of feeding 20 kittens with 250g meat samples from cattle carcasses and their feces examined daily for identification of *T. gondii* oocysts using concentration floatation technique with saturated NaCl solution (sp gr. 1.15). Sporulation of the recovered *T. gondii* oocysts were done and mice were experimentally infected with the recovered sporulated oocysts by oral inoculation (1000 oocysts / mouse) and After 6 - 8 days, the peritoneal exudates of these injected mice were examined to obtain the tachyzoites of *T. gondii*.

### 4. Preparation of parasitic and bacterial antigens:

**a. *C. bovis* antigen:** Crude whole cyst antigens were prepared by homogenization of cysts in phosphate buffer saline (PBS) and centrifuged at 10,000 g for 30 min. following the method of Cheng and Ko (1991). The supernatant was collected and its protein content was determined by the method of Lowry et al. (1951).

**b. *Toxoplasma gondii* antigen:** Local isolate antigen (LA) of *T. gondii* tachyzoites was prepared using the method described by Waltman et al. (1984). Briefly, tachyzoites were repeatedly freeze and thawed to rupture the parasite wall, sonicated and centrifuged at 12,000 rpm for 45 min at 4°C. The supernatants were separately collected and its protein content was determined by the method of Lowry et al. (1951).

**c. *E. coli* and *Salmonella* antigens:** Whole cell protein antigens of the isolated and identified *E. coli* and non typhoid *Salmonella* from meat samples were prepared according to Khan et al. (1996). Overnight bacterial culture of each strain in brain heart infusion broth was harvested by centrifugation; washed with phosphate buffer saline (PBS), pH 7.2 and resuspended in sample buffer (6.25 mM Tris {pH 6.8}; 2% SDS; 5% B-Mercaptoethanol; 10% glycerol

and 0.002% bromophenol blue). The sample was boiled in a boiling water bath for 10 min. Protein

### 5. Elisa:

The optimum antigen, serum and conjugate concentrations were determined by checkerboard titration and test procedures were carried out according to Lind et al. (1997) with little modifications. The cut off values of optical density (OD) were calculated according to Hillyer et al. (1992).

### 6. Rabbit immunization:

Two white New-Zealand rabbits were immunized with 400 µg protein per rabbit of each of the prepared parasitic and bacterial antigens emulsified in equal volumes of Freund's complete adjuvant, and two rabbits were kept as control. Booster injections were administered at 14, 21 and 28 days post-immunization in Freund's incomplete adjuvant (Alkarmi and Faubert, 1985). Serum samples were collected 7 days after the last booster injection.

### 7. SDS-PAGE and immunoblot:

SDS-PAGE of the prepared crude parasitic and whole cell protein bacterial antigens was carried out as described by Laemmli (1970) using pre-stained high molecular weight marker. The electrophoresed proteins were transferred to a nitrocellulose membrane and the immunoblotting was done according to Towbin et al. (1979).

### 3. Results

Microbiological examination of meat samples revealed that 18 out of 108 (16.67%) gave cultures of microorganisms which were as follows; 11 cows (10.19%) and 7 buffaloes (6.48%). The identified bacterial isolates were non typhoid *Salmonella* (*S. enteritidis* and *S. typhimurium*) (50%) (cows 38.89 and buffaloes 11.11%), *E. coli* (38.89%)(cows 33.33 and buffaloes 5.56%) and

concentration was determined according to Lowry et al. (1951).

*Mycobacterium bovis* (1.11%)(cows 1.11 and buffaloes 0%) (Table 1). Three out of the *E. coli* isolates (16.67%) were identified as *E. coli* O157: H7.

Parasitic examination showed that 34.26% of the collected meat samples were infected with parasites which were as follows; 31 cows (28.70%) and 6 buffaloes (5.56%). The liberated parasites were *C. bovis* (51.35%) (cows 37.84 and buffaloes 13.51%) and *T. gondii* (48.65%) (cows 18.92 and buffaloes 29.73%)(Table 2).

ELISA results showed that seroprevalence of *T. gondii* in human, cows and buffaloes were 47%, 22.7 and 38.42%, respectively.

The electrophoretic profile of the local antigen (LA) of *T. gondii* tachyzoites and *C. bovis* antigens consists of 12 (137-25.1 KDa) and 12 (185.32-24.977 KDa) protein bands, respectively (Fig. 1& 3). SDS-PAGE of the whole cell protein antigens of *E. coli* and Non typhoid *Salmonella* consists of 10 (182.01, 169.49, 144.90, 108.05, 89.032, 72.558, 60.324, 47.803, 28.312 and 18.392 KDa) and 11 (182.01, 168.20, 129.22, 91.967, 75.325, 60.955, 47.720, 43.905, 27.068, 20.91 and 14.691 KDa) polypeptide bands, respectively (Fig. 5).

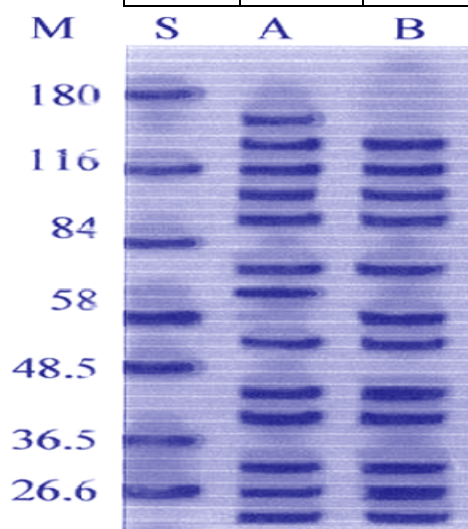
The immunoblot profile of LA of *T. gondii* tachyzoites and *C. bovis* antigens probed with the homologous rabbit hyper immune serum showed 6 (158, 111, 102, 86, 55 and 33 KDa) and 7 (167.82, 137.32, 88.839, 66.859, 59.851, 54.660 and 48.480 KDa) reactive bands, respectively (Fig. 2 & 4). The Immunoblotting profiles of *E. coli* and non typhoid *Salmonella* probed with the homologous rabbit hyper immune serum displayed 6 (182.01, 144.90, 72.558, 60.324, 28.312 and 18.392 KDa) and 3 (91.967, 60.955 and 20.031 KDa) immunoreactive bands, respectively (Fig. 6).

**Table 1: Results of bacteriological examination of meat samples**

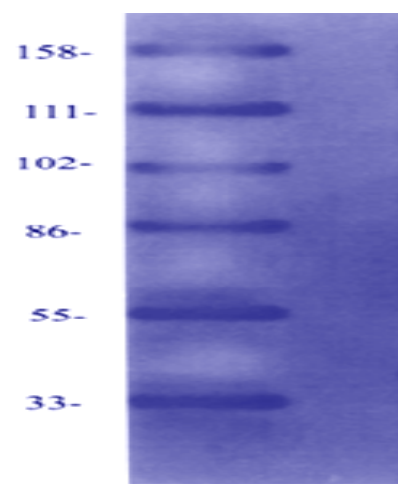
Meat samples	Total samples	positive samples	(%) of positive samples	Non typhoidal <i>Salmonella</i>	(%) of positive samples	<i>E. coli</i>	(%)of positive samples	<i>M. bovis</i>	(%)of positive samples
Cow	88	11	10.19	7	38.89	6	33.33	2	1.11
Buffalo	20	7	6.48	2	11.11	1	5.56	-	-
Total	108	18	16.67	9	50	7	38.89	2	1.11

**Table 2: Results of parasitological examination of meat samples**

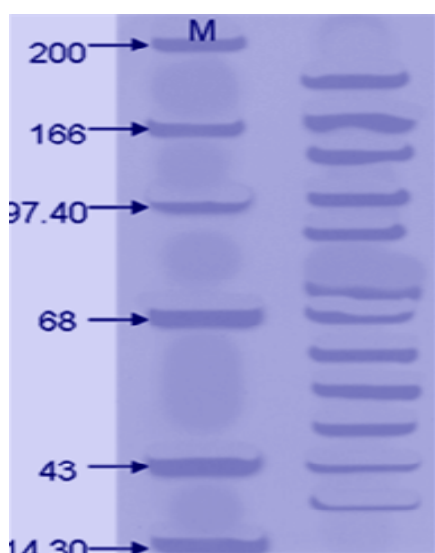
Meat samples	Total samples	positive samples	(%) of positive samples	<i>C. bovis</i>	(%) of positive samples	<i>T. gondii</i>	(%) of positive samples
Cow	88	31	28.70	14	37.84	7	18.92
Buffalo	20	6	5.56	5	13.51	11	29.73
Total	108	37	34.26	19	51.35	18	48.65



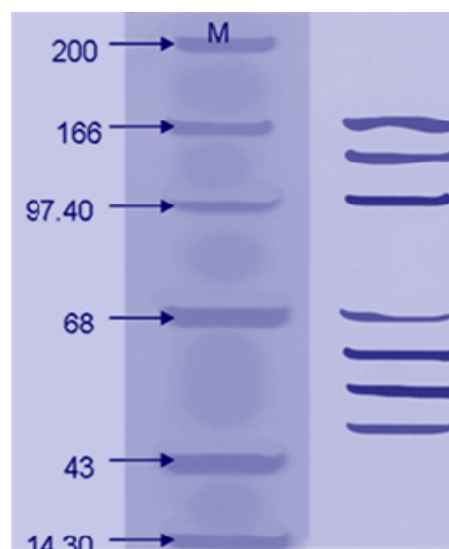
**Fig. (1):** Comparative electrophoretic profiles of *T. gondii* antigens: RH (lane A) and local strain (lane B). Standard molecular weight marker (lane S).



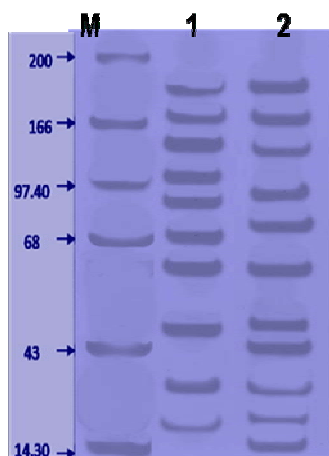
**Fig. (2):** Immunoreactive bands of *T. gondii* local strain antigen identified by rabbit hyperimmune serum using immunoblot.



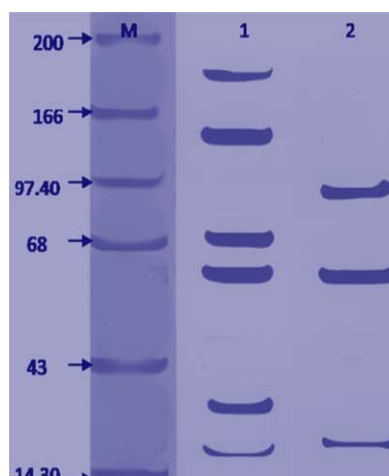
**Fig. (3):** SDS-PAGE of *C. bovis* antigen. Lane(M): Standard molecular weight marker.



**Fig. (4):** Western blot showing reactivity of *C. bovis* with rabbit hyperimmune serum. Lane (M): Standard molecular weight marker.



**Fig. (5): SDS-PAGE of whole cell protein antigens of *E. coli* (1) and non typhoid *Salmonella* (2), lane (M): Standard molecular weight marker.**



**Fig. (6): Western blot showing reactivity of whole cell protein antigens of *E. coli* (1) and non typhoid *Salmonella* (2) with rabbit hyper immune serum, lane (M): Standard molecular weight marker.**

#### 4 .Discussion

Diagnosis of food borne bacterial diseases is carried out through the isolation of organisms from implicated food or feces and detection of toxins. Serologic testing is an important method for detecting parasitic infections, and includes immunofluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), competitive-inhibition ELISA, Western blotting, and direct agglutination test (DAT) (Jenkins et al., 2002; Huang et al., 2004).

In the current study, *E. coli* (38.89% as 33.33 cows & 5.56% buffaloes) and *E. coli* O157:H7 (16.67%) were identified. Stampi et al. (2004) detected *E. coli* and *E. coli* O157 in 30.2 and 2% of the 149 bovine meat samples examined, respectively. Fairbrother and Nadeau (2006) isolated *E. coli* O157 from 14.3% of bovine carcasses. Also, non typhoid *Salmonella* (50% as 38.89 cows & 11.11% buffaloes) was isolated. Beach et al. (2002) recorded isolation rate of salmonellae of 19% from carcass samples of cattle. While, Al-Lahham et al. (1990), Branham et al. (2005) and Maharjan et al. (2006) reported isolation rate of *Salmonella* (6%), *E. coli* and *Salmonella* spp. in cattle (1.25%) and *Salmonella* from raw buffalo meat (13.5%), respectively. Phillips et al. (2008) isolated *E. coli* and *Salmonella* from 17.8% and 1.1% of ground beef samples, respectively. *E. coli* O157 was recovered from 0.3% of samples.

Microbiological examination of meat samples revealed that 1.11% was infected with *M. bovis* (cows 1.11 and buffaloes 0%). Teklul et al.

(2004) found that of the 751 carcasses examined, 4.5% were found to have tuberculous lesions. Routine abattoir inspection detected only 29.4% of the carcasses with visible lesions. In addition, *M. bovis* was isolated from a carcass that presented no gross tuberculosis lesions. The low sensitivity of routine abattoir inspection demonstrates that existing necropsy procedures should be improved. Tadayon et al. (2006) recorded that 1.43 and 72.22% of the examined specimens from cows and buffaloes, respectively were positive for *M. bovis*.

In this study, parasitic examination showed that 34.26% (37/108) of the collected meat samples were infected with parasites (cows 28.70 and buffaloes 5.56%). The liberated parasites were *C. bovis* (51.35%) (cows 37.84 and buffaloes 13.51%) and *T. gondii* (48.65%) (cows 18.92 and buffaloes 29.73%). Wanzala et al. (2003) found cysticerci in 12/25 (48%) and 24/25 (96%) of infested cow by inspection and total dissection, respectively and indicated that except for the dead, degenerate or calcified cysticerci a careless meat inspector will most likely miss out quite a number of viable cysticerci, and be passed on for human consumption, becoming the source of bovine cysticercosis. Megersa et al. (2010) reported that of the total of 500 inspected cattle, 22 animals had varying number of *C. bovis* giving an overall prevalence 4.4% (22/500). On the other hand, Pearse et al. (2010) reported that of the 23 samples submitted, none was positive for *C. bovis* by either diagnostic method.

Although meat examination revealed that

18.92 and 29.73% of the examined cow and buffalo meat were infected with *T. gondii* cysts, ELISA results showed that the seroprevalence of *T. gondii* in cows and buffaloes were 22.7% and 38.42%, respectively. The higher incidence of *T. gondii* in the tested animals by ELISA is probably attributed to cross-reactivity, particularly with the use of crude extracts not purified antigens. Lower seroprevalence (8.8%) was recorded in buffaloes examined by Navidpour and Hoghooghi (1998). On the other hand, Selvaraj et al. (2007) found that sera of 99 she-buffaloes slaughtered showed 100% seropositivity for antibodies to *Toxoplasma gondii* by modified direct agglutination test at a dilution of 1:200. Also, our results recorded 47% prevalence of toxoplasmosis in human. Ibrahim et al. (2009) detected anti-*T. gondii* antibodies in pregnant women (51.49%) and cattle (10.75%) examined at Northern Egypt and stated that the high prevalence of toxoplasmosis in cattle affects the development of the livestock industry and is also an important infective source for human infection in Egypt.

In the present investigation, the electrophoretic profile of *C. bovis* antigen displays 12 polypeptide bands located at 185.32- 24.97 KDa. Similar results were recorded by Kandil et al. (2003).

The whole cell protein electrophoretic patterns of *E. coli* and non typhoid *Salmonella* displayed 3 common bands at 182, 60 and 47 KDa. The non typhoid *Salmonella* whole cell protein SDS-PAGE profile showed a 20.91 KDa protein band specific for *Salmonella*. Gupta et al. (2005) compared the protein profiles of selected *Salmonella* serovars with *E. coli* to identify genus specific protein(s) for *Salmonella* and stated that a protein of molecular weight 20.89 KDa was found in all *Salmonella* serovars, but not in *E. coli* suggesting its genus specific attribute.

Our results showed that the electrophoretic profile of the whole cell protein antigen of *E. coli* consisted of 10 proteins with molecular weight of 182-18.392 KDa. While, Uçan et al. (2005) reported that SDS-PAGE of the whole cell protein extracts of *E. coli* produces patterns containing 26 to 35 discrete bands with molecular weights of 6500-200 KDa. Also, Nawal (2008) found that the electrophoretic profile of the whole cell protein antigen of *E. coli* contains 10 protein bands with molecular weight range 775- 12 KDa.

In the present study, the immunoblotting profiles of *E. coli* and non typhoid *Salmonella* probed with its homologous rabbit hyper immune serum displayed 6 (182.01, 144.90, 72.558, 60.324, 28.312 and 18.392 KDa) and 3 (91.967, 60.955 and 20.031 KDa) protein bands, respectively. While, Nawal (2008) reported that the immunoblot fingerprinting of *E. coli* and non typhoid *Salmonella* whole cell protein

antigens probed with human serum showed 6 ( 75.00, 70.36, 45.15, 43.00, 25.83 and 14.965 KDa) and 6 (138.48, 56.04, 46.36, 25.83, 20.91 and 14.00 KDa) protein bands, respectively.

In the current study, the immunoreactive profiles of *C. bovis* (167.82, 137.32, 88.839, 66.859, 59.851, 54.660 and 48.480 KDa) & *T. gondii* local tachyzoite (158, 111, 102, 86, 55 and 33 KDa) and that of *E. coli* (182.01, 144.90, 72.558, 60.324, 28.312 and 18.392 KDa) & non typhoid *Salmonella* (91.967, 60.955 and 20.031 KDa) antigens showed common bands at 55 and 60 KDa, respectively. So, it can be concluded that beside immunoblotting helps in the identification of the different bacterial and parasitic strains, it can also detect common (cross reactive) immunoreactive epitopes between the antigens of the respective strains which may be beneficial in making shared vaccines.

It can be concluded that despite the recent advances in food pathogen detection, there still exist many challenges and opportunities to improve the current technology. Immunoblotting can help in the identification of pathogens and finding common immunoreactive epitopes between them with the possibility of manufacturing common cross protective vaccines.

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