

Arch Lebensmittelhyg 67,
79–86 (2016)
DOI 10.2376/0003-925X-67-79

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ISSN 0003-925X

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Summary

Zusammenfassung

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Food safety and risk management of beef and beef products in Pakistan

Lebensmittelsicherheit und Risikomanagement bei Rindfleisch und Rindfleischerzeugnissen in Pakistan

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In this paper, biological hazards associated with beef in Pakistan are identified along the food chain and ranked according to the possibility i) to detect the hazard during meat inspection, and ii) that the risk would increase during cold storage of beef or that it would decrease during thermal meat processing. It appears that meat inspection has limited potential to control the hazards. Since the demand for beef (including 'novel' beef products) is expected to rise particularly in the metropolitan areas of Pakistan, it is increasingly important that handling and preparing beef dishes is done in such a way as to prevent hazardous agents from spreading and multiplying, or to reduce them to an acceptable level. The clear formulation of- and strict adherence to Good Manufacturing and Hygiene Practices – as well as the provision of information to the consumer on residual risks that may persist – is primarily the responsibility of food business operators. More intensified attempts to educate the consumers on how to reduce or eliminate these risks need to be undertaken.

Keywords: Pakistan, beef, biological hazards, risk management, risk ranking

In dieser Arbeit werden die mit Rindfleisch in Pakistan assoziierten biologischen Gefahren entlang der Lebensmittelkette identifiziert und gereiht. Die Reihung erfolgt nach der Wahrscheinlichkeit, die Gefahr bei der Fleischuntersuchung zu erkennen und der Wahrscheinlichkeit der Änderung des Risikos während der Kühlung des Fleisches und während der Zubereitung (Erhitzung). Es zeigte sich, dass die Schlachtier- und Fleischuntersuchung nur begrenzte Möglichkeiten zur Beherrschung dieser Gefahren bietet. Da der Rindfleischverzehr (einschließlich „neuartiger“ Produkte) insbesondere im städtischen Bereich in Pakistan steigen wird, ist es umso wichtiger, dass der Umgang mit und die Zubereitung von Rindfleischgerichten so erfolgen, dass der Verbreitung und Vermehrung von Noxen vorgebeugt wird bzw. dass Gefahren auf ein annehmbares Niveau verringert werden. Die klare Formulierung von Leitlinien für eine Gute Hygiene Praxis und deren strikte Befolgung sowie eine Information der Verbraucher über Restrisiken sind eine primäre Verantwortung der Lebensmittelunternehmer. Eine intensivere Aufklärung der Konsumenten ist nötig, um die biologischen Gefahren zu verringern oder zu eliminieren.

Schlüsselwörter: Pakistan, Rindfleisch, biologische Gefahren, Risikomanagement, Reihung der Risiken

Introduction

In developing countries, meat constitutes a small part of the human diet, but in growing economies, the demand of meat on the domestic market is going to rise. In addition, meat export can have a significant economic impact for the development of local meat industry. In a previous paper (Nauman et al., submitted), we provided a review of the supply chain for beef and beef products in Pakistan. In the present paper, the characterization of the beef chain is linked to the microbial food borne hazards identified in Pakistan, and an assessment of consumer exposure and of the efficacy of control measures is conducted, to allow ranking of beef borne microbiological risks.

A recent review on food safety issues in Pakistan (Akhtar, 2013) has primarily dealt with non-meat products. In contrast, the present review focusses on i) identifying the hazards associated with meat production, processing and consumption, and ii) critically evaluating how the risks associated with major pathogens are currently dealt with.

Biological hazards prevalent in the beef chain in Pakistan and neighbouring countries, with a focus on food safety

The demand for fresh natural food, free from diseases, pesticide, fertilizers, allergens, additives, preservatives and GMO's, is increasing in the developed as well as the developing world. Thus, food security is closely linked to food quality and food safety requirements. Health problems related to unsafe food are known since first history was recorded. Today, more than 200 diseases are known, which are transmitted through food (Mead et al., 1999). In this context, Good Management and Hygiene practices are vital as this will not only help to protect the health of consumers but also provide opportunities to access the export markets.

Major biological hazards associated with meat include spoilage bacteria, pathogenic bacteria (e. g. *Escherichia coli*, *Salmonella* serovars, *Brucella*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Coxiella burnetii*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium* spp., *Mycobacterium bovis*, *Streptococcus* spp.), viral pathogens and parasites (e. g. *Cryptosporidium parvum*, *Giardia duodenalis*, *Cysticercus bovis*, *Onchocerca* spp., *Taenia saginata*, *Toxoplasma gondii*) (Sofos, 2013). Contamination is hardly avoidable as these microorganisms are present on animals and in the environment (Stoica et al., 2014). While some microorganisms thrive in niches on/in the otherwise healthy animal, others are not a natural part of the healthy animal's 'microbial association' but – along the long supply chain from farm to fork including handling, slaughtering, processing, storage, equipment & utensils, workers and transportation – may be transmitted to- and contaminate foods of animal origin (Fig. 1). As is the case for many other developing countries, documented data about food safety, food quality and the incidence of food borne disease are hardly available for Pakistan. Too few (and even more so country-specific) studies on biological zoonotic hazards are undertaken and subsequently reported while the public sector appears to be completely silent on these issues.

As regards biological hazards and risks associated with meat production in Pakistan, it is even more difficult to

retrieve the relevant data. A major objective of this paper was therefore to report on observations that would allow getting a clearer picture of some of these hazards and risks and to evaluate if/how these are currently managed in Pakistan.

Biological hazards – on farm

Both in extensive and intensive beef production systems, exposure to biological hazards may occur at any stage (FSANZ, 2013a). These include common food borne pathogens such as *Campylobacter* spp., *Clostridium* spp., pathogenic *E. coli*, *Listeria monocytogenes*, *Salmonella* spp., *Yersinia enterocolitica*, *Mycobacterium bovis*, *Brucella abortus*, *Yersinia pseudotuberculosis*, *Mycobacterium avium* ssp. *paratuberculosis*, *Cryptosporidium parvum* and *C. muris*, and *Sarcocystis hominis* (Drexler, 2003). As recently reviewed by Buncic et al. (2014), microorganisms are present in the animal's gastrointestinal tract and on its skin, i.e. the natural and external environment for biological hazards. From these environments, biological hazards may be spread to the surfaces of carcasses and primal cuts during slaughter and processing.

Hussain et al. (2008a) detected *Brucella* antibodies in 8 % of the cattle, 6.92 % of buffalo and 11 % of human samples in Pakistan. Other authors report sero-prevalences

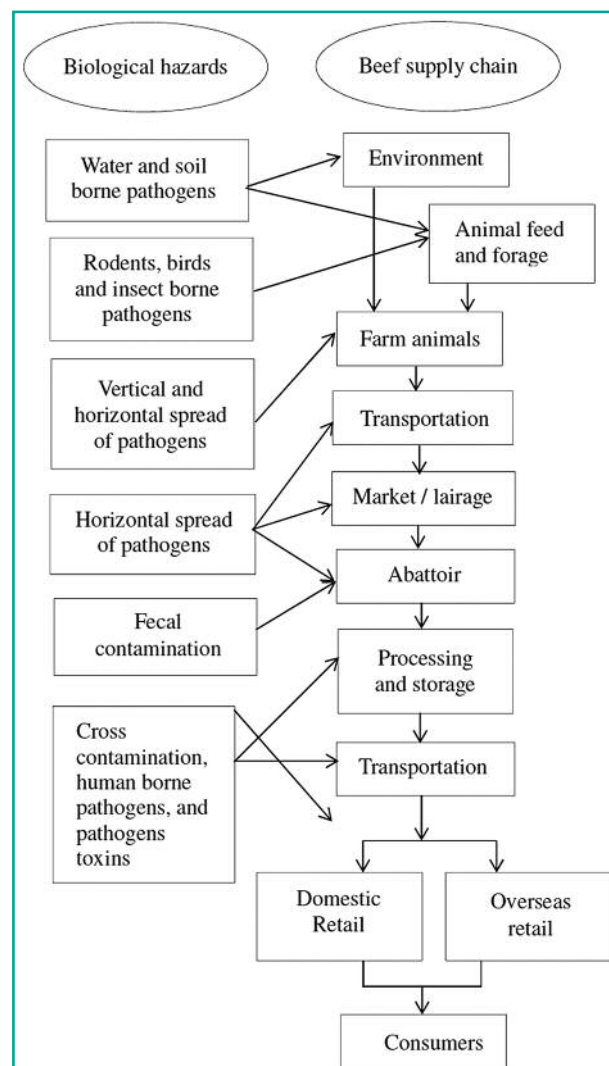


FIGURE 1: Schematic diagram of the beef supply chain and potential points of biological hazards entry (adapted from Buncic, 2000).

of brucellosis as high as 14.70 % in cattle at government farms, and 18.53 % in cattle at various private livestock farms (Nasir et al., 2004). More recently, Waqas et al. (2015) detected brucellosis in 11 % of cattle.

Aftab et al. (2012) reported that in Peshawar, Pakistan, hides of 89.6 % animals were contaminated with *Salmonella* spp. Masood et al. (2013) studied the prevalence of *Cryptosporidium* spp. in Lahore, Pakistan and found that 10.5 % of cattle were infected, the highest infection rate (20.55 %) being observed in animals held at government farms.

Some epizootic and trans-boundary diseases in beef animals such as Foot and Mouth Disease and Rinderpest (RP) have serious economic consequences in terms of costs of treatment/vaccination, production loss, meat quality and product availability (Hussain et al., 2008b). Pakistan has been declared free from RP in 2007 (FAO, 2011) whilst FMD is still prevailing, resulting in estimated losses of \$ 82 million (Raines, 2011). Data about other zoonotic hazards could not be retrieved.

Biological hazards – abattoir level

At this stage in the supply chain, meat is exposed to many internal and environmental biological hazards. These are introduced during bleeding, trimming, bunging, evisceration and carcass washing by fecal leakage, contaminated utensils, knives and other cutting tools (FSANZ, 2013a). Major microorganisms of concern include: pathogenic *E. coli*, *Salmonella* spp., *Staphylococcus* spp., *Enterobacteriaceae*, *L. monocytogenes*, *Mycobacterium avium* subsp. *paratuberculosis*, *F. hepatica*, *F. gigantica*, *Giardia lamblia*, *Taenia saginata* and *Toxoplasma gondii*.

Ayaz et al. (2014) collected samples from the Khyber Pakhtunkhwa (KPK) province, Pakistan and found that 8 % of liver samples of cattle and buffaloes were infested with liver fluke. In a similar study the prevalence of fascioliasis was found to be 17.61 %, of which *F. hepatica* was 5.7 % and *F. gigantica* 9.83 %, with mixed infections of these two species being revealed in 2.08 % of the livers examined (Hayat et al., 1986). In another study, Nauman et al. (2013) reported that 2.1 % of cattle and 4.7 % of buffaloes slaughtered in Lahore were infected with *Cysticercus bovis*. Gulf-Cooperation-Council (GCC) exporting countries are seriously concerned about the *Cysticercus* parasite that is responsible for a major percentage of all carcass rejections. As regards occupational disease, Fatima (2010) reported that 21.7 % serum samples from slaughter house workers in Lahore, Pakistan, carried anti-*Brucella* antibodies.

Tuberculosis and Paratuberculosis were diagnosed in 1 % and 2.5 % of 200 cattle slaughtered in Faisalabad, Pakistan (Waqas et al., 2015). Aftab et al. (2012) detected *Salmonella* in all of a total of 100 samples from cattle hides, carcass surfaces and beef cuts sampled along the beef chain in Peshawar, Pakistan. As regards pathogenic *E. coli*, no data were found for Pakistan, but in Amman, enterotoxigenic *E. coli* O157:H7 were detected by PCR in 8.3 %, 10 %, and 7.8 % of faeces, hides and carcasses of calves, respectively (Osaili et al., 2013).

Khan et al. (2010) analysed the Echinococcosis problem in cattle by studying a large cattle population slaughtered in an abattoir in Lahore and reported a prevalence rate of 4.27 % in females and 1.20 % in males; the infection frequency order in the various organs was as follows: lungs > liver > spleen > abdominal cavity. Similar results are reported by Anwar et al. (2000). Zahida et al. (2014) repor-

ted that 10 % of buffaloes in Multan (Punjab region) are carrying *Echinococcus*.

Biological hazards – storage/processing/distribution

Studies carried out in different regions of Pakistan (particularly in large cities) reveal the current hygiene and processing conditions that relate to food safety. In particular, pathogenic *E. coli*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus*, *Enterobacter* and *B. cereus* could be detected (FSANZ, 2013a). Hassan et al. (2010) examined meat and meat contact surfaces in retail shops in Karachi, Pakistan and (from 342 meat samples and 208 surface swabs) isolated 550 potential pathogenic bacteria including *E. coli* O157:H7, *Listeria*, *Enterobacter*, *Staphylococcus aureus*, *Salmonella* Enteritidis, *Brucella* and *Shigella* with total aerobic counts ranging from 10⁸ to 10¹⁰ CFU/g. In a similar study on the prevalence of enterohemorrhagic *E. coli* (EHEC) in different meat sources, Hussain et al. (2013) found an overall prevalence of 11.8 % of EHEC in different types of meat (beef, mutton, chicken) in Kohat, Pakistan.

Ahmad et al. (2013) detected *Salmonella*, *Staphylococcus* and *E. coli* in beef samples both from the abattoir and from retail shops in Lahore and found 20 % and 35 % samples contaminated with *Salmonella*, 55 % and 70 % with *Staphylococcus* and 40 % and 75 % with *E. coli*, respectively. A 100 % prevalence for *Salmonella* has been reported by Aftab et al. (2012) for Peshawar, Pakistan. Hussain et al. (2007) reported the presence of *Campylobacter* on 10.9 % and 48 % of beef and chicken meat samples, respectively in three big cities of Punjab (Faisalabad, Lahore and Islamabad), Pakistan. Kausar and Hafza (2010) recovered *E. coli* O157:H7 from 83 % samples of beef taken from different localities. Finally, Nawaz et al. (2014) screened *E. coli* strains in stool samples from Karachi, Pakistan, for the presence of shiga toxin 1 and shiga toxin 2 genes of enterohaemorrhagic *E. coli* (EHEC) and enterotoxigenic *E. coli* (ETEC), and reported that 16 out of 37 strains carried one or more type of toxin genes. Although the prevalence of *Staphylococcus aureus* can exceed 50 % (Ahmad et al., 2013), the levels of this pathogen are not necessarily high, e. g. Hamida et al. (2010) report a maximum concentration of 20 CFU/g in beef patties.

In search of antibacterial sensitivity, Khatoun et al. (2014) collected beef samples from Lahore, and found that 85 % samples were positive for major food borne pathogens, as *Staphylococcus aureus*, *Bacillus cereus*, *E. coli* and *Campylobacter jejuni*. Interestingly, *S. Typhi* was also detected on beef, which indicates contamination of meat by workers or food handlers excreting this human-specific *Salmonella* serovar (USFDA, 2014). Isolates were tested against fluoroquinolones and aminoglycosides, with the following order of resistance prevalence: moxifloxacin > ciprofloxacin > levofloxacin > and tobramycin > amikamycin > neomycin, respectively.

Material and methods

In order to assess the microbiological safety of traditional Pakistani beef dishes, we prepared Keema and Bhuna Gosht according to the description of Bhandare (2014). Temperature inside meat was measured by thermologgers (Thermochron®, Maxim Semiconductors, USA).

The impact of “unsafe” food handling and preparation practices at population level was estimated using the MS-Excel add-in “Risk Ranger” (Ross and Sumner, 2002).

Finally, we attempted to rank the biological hazards identified in the previous sections using a decision tree derived from those recently developed in the framework of EFSA scientific opinions on meat inspection (EFSA, 2011; 2013). First, a “long list” of hazards was composed based on the data relevant for Pakistan as reviewed in the previous sections, and on recent hazard analyses conducted by the EFSA (2013). The identified hazards were further characterised according to the decision tree shown in Fig. 2 and subsequently qualitatively ranked. To this end, the storage and food preparation habits including time-temperature categorization were considered.

Results and discussion

Food preparation in the kitchen and meat consumption practices

Almost all recipes for domestic or restaurant meat preparations suggest a) heating at temperatures ranging from 75 °C to 180 °C aiming at ultimate internal temperatures of 65±2 °C (Bhandare, 2014) and b) extended cooking times (60–70 min). In a laboratory trial we prepared Keema and Bhuna Gosht using such recommended procedures and recorded the internal temperature of the product. Indeed, after 4 min. of heating, 86 °C were reached in a minced meat preparation (Keema) and 77 °C inside the meat chunks (Bhuna Gosht); these temperatures were held for 45 min, and the cooling down of these meals to ambient temperature (25 °C) took 25 min.

Hence, it may be assumed that most food borne hazards are likely to be destroyed or inactivated and that (immediately after cooking) – according to international references (FSIS, 2015, Government of Canada Health Authorities, 2014, New South Wales Food Authority, 2014) – such time/temperature profiles generally yield microbiologically “safe” beef.

However, all heated products in the Pakistani domestic cuisine have in common that they A) are either left to cool down for several hours to ambient temperature (25–45 °C) before being served at noon, or B) may even be cooled down overnight. Such practices have been estimated to be common in more than 50 % of Pakistani homes (Bryan, 2000), which raises concern, e. g. on staphylococcal and bacillus toxin formation (FSANZ, 2001). Bryan et al. (1992) reported that, although following procedure-A) renders foods that generally have mesophilic aerobic colony counts less than 10,000 CFU/g, procedure-B) ultimately results in counts as high as 10⁶–10⁹ CFU/g, and that coliform bacteria and *Clostridium perfringens* were isolated from 77 % and 18 % samples, respectively; only three of 28 samples contained *Bacillus cereus*, whilst *Salmonella* was not recovered from any of 28 samples. These findings may also be associated with cross contamination via addition of spices and garnishment (e. g. mint or coriander leaves) as reported by Vij et al. (2006), or

recontamination via knives, cutting boards and hands of workers.

In urban busy life where time for proper cooking generally fails, the consumer increasingly relies on convenience foods (sometimes based on exotic recipes) and Ready To Eat (RTE) products. This applies to meat dishes also. Many food companies enter this market and distribute such products, which vary significantly both in quality and price. The various products’ formulations are based on traditional (and market-driven) taste preferences. Due to high temperature changes over the various seasons, many of these meat products (that are supposed to be ‘shelf stable’) are sold in frozen form. However, as a result of frequently occurring shortages of electricity, these food items may occasionally indeed harbour pathogenic organisms and may be the cause of severe food borne illness.

A risk based approach to grade biological hazards in the meat chain: ranking of hazards and assessment of impact of food handling practices

The common Pakistani meat preparation practices described above allow the assumption – even in the absence of sufficient reliable prevalence data – that a wide array of pathogens will be present in (raw or processed) beef placed on the market. The ranking of these hazards using an EFSA-derived decision tree (Fig. 2) is presented in Table 1.

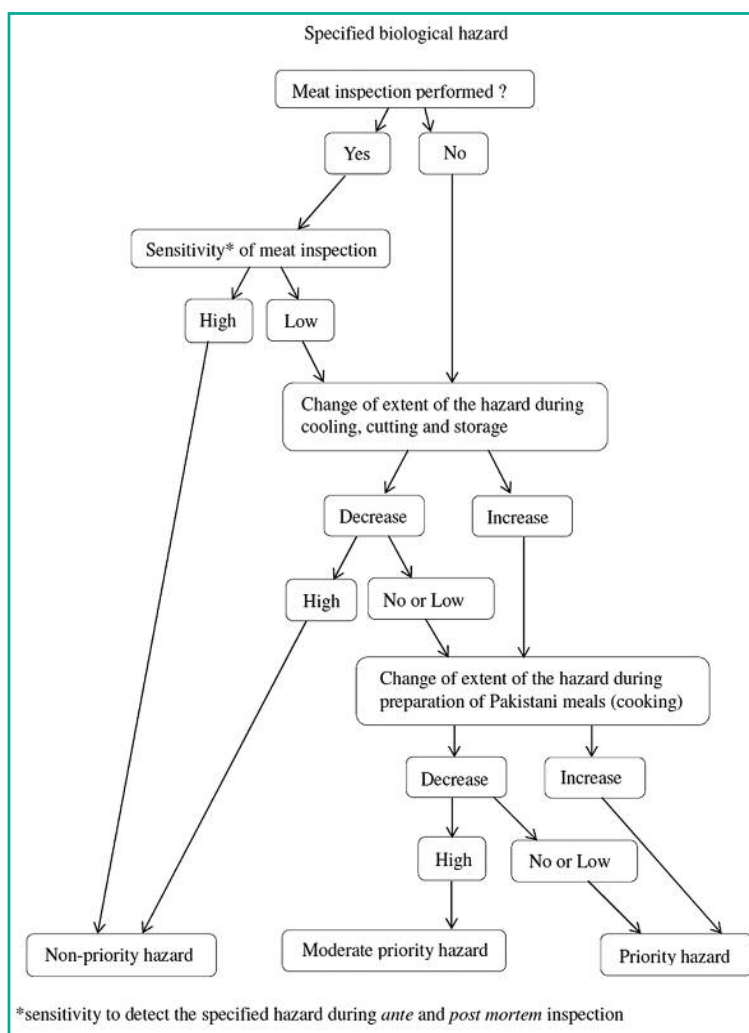


FIGURE 2: Generic decision tree to rank biological hazards in the beef chain.

TABLE 1: Outcomes of risk ranking for major biological hazards prevalent in beef prepared according to the traditional Pakistan cuisine.

Type	Bovine meat borne transmission?	Hazard detectable during meat inspection? (H/L*)	Risk increase during chilled storage?	Risk decrease during thermal treatment?	Rank
- Virus -					
<i>Lyssavirus (rabies)</i>	No / Contact, primarily through animal bites (EFSA, 2013)	Yes (L) (EFSA, 2013)	No (Bourhy, 1998)	Yes (Kopcha, 2010)	Moderate
<i>Encephalitis virus (TBEV from family Flaviridae)</i>	No / Vector-borne (EFSA, 2013)	Yes (L) (EFSA, 2013)	No (Prince and Prince, 2001)	Yes (A.H.A 2003)	Moderate
- Prion -					
Transmissible spongiform encephalopathy	Yes (EFSA, 2015)	No (CFIA, 2013)	No (EFSA, 2014)	No (Brown et al., 2000)	High
- Bacterium -					
Pathogenic <i>E. coli</i> / VTEC	Yes (EFSA, 2013)	No (EFSA, 2013)	No (EFSA, 2014)	Yes (Stringer et al., 2000)	Moderate
<i>Brucella abortus</i>	No / By contact (EFSA, 2013)	Yes (L) (EFSA, 2013)	No (Abbas and Talei, 2010)	Yes (CDC, 2012)	Moderate
<i>Salmonella</i> spp.	Yes (EFSA, 2013)	No (EFSA, 2013)	Yes (EFSA, 2014)	Yes (FSIS, 2015)	Moderate
<i>Streptococcus</i> spp.	No / Contact (EFSA, 2013)	Yes (L) (EFSA, 2013)	No (Voidarou et al., 2007)	Yes (Hvalbye et al., 1999)	Moderate
<i>Staphylococcus aureus</i>	Yes (EFSA, 2013)	Yes (L) (EFSA, 2013)	No (Albrecht, 2005b)	Yes (Heinz and Hautzinger, 2007)	Moderate
<i>Listeria monocytogenes</i>	Yes (EFSA, 2013)	No (EFSA, 2013)	Yes (EFSA, 2014)	Yes (EFSA, 2013)	Moderate
<i>Mycobacterium bovis</i>	No / by Aerosol (EFSA, 2013)	Yes (L) (EFSA, 2013)	No (Cressey et al., 2006)	Yes (Cressey et al., 2006)	Moderate
<i>Campylobacter jejuni</i>	Yes (CDC, 2014)	No (EFSA, 2013)	No (ICMSF, 1996)	Yes (Heinz and Hautzinger, 2007)	Moderate
<i>Yersinia enterocolitica</i>	No / Food borne (EFSA, 2013)	No (EFSA, 2013)	Yes (EFSA, 2014)	Yes (Bari et al., 2011)	Moderate
<i>Bacillus cereus</i>	Yes (EFSA, 2013)	No (EFSA, 2013)	No (Albrecht, 2005a)	Yes (Albrecht, 2005a)	Moderate
<i>Clostridium</i> spp.	Yes (EFSA, 2013)	No (EFSA, 2013)	No (USFDA, 2011)	Yes (USFDA, 2011)	Moderate
<i>Shigella</i> spp.	No / Contact (FSANZ, 2013b)	No (EFSA, 2013)	No (FSANZ, 2013b)	Yes (FSANZ, 2013b)	Moderate
<i>Bacillus anthracis</i>	Yes (EFSA, 2013)	Yes (L) (EFSA, 2013)	No (EFSA, 2005)	Yes (EFSA, 2005)	Moderate
- Parasite -					
<i>Cryptosporidium parvum</i> and <i>C. muris</i>	No / Food and water borne (EFSA, 2013)	No (EFSA, 2013)	No (EFSA, 2014)	Yes (Moriarty et al., 2005)	Moderate
<i>F. hepatica</i> and <i>gigantica</i>	No / Contaminated environment (EFSA, 2013)	Yes (H) (EFSA, 2013)	No (EFSA, 2014)	Yes (PHAC, 2001)	Moderate
<i>Giardia lamblia</i> and <i>duodenalis</i>	No / Food and water borne (EFSA, 2013)	No (EFSA, 2013)	No (EFSA, 2014)	Yes (USDA, 2013)	Moderate
<i>Taenia saginata</i>	Yes (EFSA, 2013)	Yes (L) (EFSA, 2013)	No (EFSA, 2014)	Yes (Krauss et al., 2003)	Moderate
<i>Toxoplasma gondii</i>	Yes (EFSA, 2013)	No (EFSA, 2013)	No (EFSA, 2014)	Yes (Dubey et al., 2005)	Moderate
<i>Sarcocystis hominis</i>	Yes (EFSA, 2013)	No (EFSA, 2013)	No (EFSA, 2014)	Yes (Ortega and Sulaiman, 2014)	Moderate

*H(igh): sensitivity of inspection > 90 % ; L(ow): sensitivity of inspection < 90 %

All listed hazards – with the exception of prion disease – are ranked as “moderate” and associated risks would appear to be manageable. However, even when proper meat inspection would be consistently conducted (which unfortunately is not always the case), most of the hazards will persist in the beef chain, and they will be reduced or eliminated only provided suitable preparation techniques are applied. This essential information needs to be conveyed to the consumer (‘risk communication’).

The cooking procedures for traditional beef dishes rely on time/temperature combinations that should be lethal for most biological hazards considered in Table 1. Yet, as stressed by EFSA (2012), essentially relying on the end-user (consumer) to eliminate prevailing risks is far from prudent, as any compromising circumstance (e. g. undercooking, recontamination, addition of contaminated garnishing, extended post-cook holding times at room temperature) may then indeed lead to food borne disease outbreaks. The relative contribution of food preparation habits to the risk of acquiring food borne disease can be estimated by Risk Ranger[®] software (Ross and Sumner, 2002). Using abovementioned data, relying on current food preparation practices, a total of 3.8×10^5 cases of food borne illness would be expected *per annum*, whereas ‘unsafe’ cooking procedures would increase that number by two log units to 3.8×10^7 .

Consequently, given the situation in Pakistan, more intensified attempts to communicate with the consumer – be it in a domestic or gastronomic setting – on the residual risks persisting in raw and processed food items is vital, even more so than is the case for the more developed world. Unless this is effectively achieved, consumer safety and the minimisation of beef borne diseases cannot be warranted.

Conclusions

In Pakistan, beef production is traditionally a side branch of keeping dairy and draught cattle, but the demand for beef and also for beef products is expected to rise at least in the urban centres. Food control systems are in place for beef export and certain branches of local food supply, but there is a lack of knowledge on the prevalence of zoonotic hazards in the beef chain, and the inspection system is not focused on the hazards of most concern. This, together with expected changes in beef production and processing is very likely to cause hazardous situations.

Under these circumstances, it is essential that handling, processing and preparation of beef products is done in such a way as to prevent hazardous agents from spreading and multiplication, or to reduce them to an acceptable level. The clear formulation of – and strict adherence to Good Manufacturing and Hygiene Practices – as well as the provision of information to the consumer on residual risks that may persist – is primarily the responsibility of food business operators. More intensified attempts to educate the consumers on domestic preparation practices that will further reduce or eliminate these risks need to be undertaken.

Acknowledgment

The first author was funded by a grant of the Higher Education Commission of Pakistan.

Conflict of interest

The authors declare that no conflicts of interest exist.

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