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Prevalence of *Cysticercus bovis* in Cattle and Buffalo in Punjab and Sindh provinces, Pakistan

Prävalenz von Cysticercus bovis in Rindern und Büffeln aus den pakistanischen Provinzen Punjab und Sindh

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Summary

Cysticercosis has a significant impact not only on public health but also on agricultural development worldwide. In order to provide an estimate of the prevalence of *Cysticercus bovis* in cattle and buffaloes in certain regions of Pakistan, 3731 cattle and 1105 buffaloes were examined in the period of April through October, 2009 in an export slaughter house in Lahore by extended meat inspection (masseter and pterygoid muscles, freed tongue, heart, diaphragm, esophagus, *M. triceps brachii*). The proportion of carcasses found to be infected with cysts was significantly different for species (cattle: 2.1 %, buffalo: 4.7 %; $P = 0.002$), gender (higher in females; $P < 0.001$) and age (higher in animals > 2.5 years of age; $P < 0.001$). In detail, the percentages were in cattle and buffaloes 2.6 % and 6.0 % for females vs. 1.9 % and 4.1 % for males, and 3.8 % and 7.4 % for animals > 2.5 years of age vs. 1.4 % and 2.8 % for animals < 2.5 years of age.

Keywords: Foodborne zoonosis, meat inspection, risk factor

Zusammenfassung

Zystizerkose hat weltweit Bedeutung für das öffentliche Gesundheitswesen und die Entwicklung der Landwirtschaft. Um eine Einschätzung der Häufigkeit des Vorkommens von *Cysticercus bovis* beim Rind und beim Büffel in bestimmten Regionen Pakistans zu erhalten, wurden in einem Exportschlachthof in Lahore 3731 Rinder und 1105 Büffel im Zeitraum April bis Oktober 2009 einer erweiterten Fleischuntersuchung unterzogen (*Mm. masseteres* und *pterygoidei*, Zunge, Herz, Zwerchfell, Speiseröhre und der *M. triceps brachii*). Hinsichtlich des Nachweises von Bandwurmfinnen gab es signifikante Unterschiede zwischen den Tierarten (Rind: 2.1 %, Büffel: 4.7 %; $P = 0.002$), Geschlecht (häufiger bei weiblichen Tieren; $P < 0.001$) und Alter (häufiger in über 2,5 Jahre alten Tieren; $P < 0.001$). Die Nachweishäufigkeit betrug beim Rind und Büffel 2.6 % und 6.0 % für weibliche bzw. 1.9 % und 4.1 % für männliche Tiere, 3.8 % und 7.4 % für über 2,5 Jahre alte Tiere bzw. 1.4 % und 2.8 % für unter 2,5 Jahre alte Tiere.

Schlüsselwörter: Lebensmittelübertragene Zoonose, Fleischuntersuchung, Risikofaktor

Introduction

In Pakistan, cattle and buffalo constitute the major source of red meat. The Punjab and Sindh provinces hold 72 % and 93 % of the cattle and buffalo livestock, respectively (Anonymous, 2013). Inspection and slaughter of animals is governed by provincial legislation, e. g. the Punjab Animals Slaughter Control Act of 1963 (Anonymous, 1963). Most of the meat is intended for the domestic market, exports accounting for roughly 1.2 % of the production. Although the latter percentage is rather small, this segment is attractive in financial terms, and Pakistani meat export is expected to rise until 2020 (OECD-FAO, 2011). For such meat, inspection is done according to the specifications of the importing country. Amongst the biological hazards relevant in red meat, cysticercosis is a major foodborne parasitic zoonosis of cattle and buffalo, with worldwide economic significance as it markedly affects food security (Basem et al., 2009). Meat from cattle and buffalo containing *Cysticercus bovis* larvae represents a major reservoir for human taeniosis (Roberts et al., 1994). In many countries, current risk management strategies still largely rely on post-mortem inspection and condemnation or additional treatment of infected carcasses, which causes significant economic losses to livestock farmers and industry (EFSA, 2005).

There are no published data on the economic losses due to partial or complete rejection of cattle carcasses due to bovine cysticercosis in Pakistan, but it is estimated that this problem causes losses up to 300 000 US\$ per annum (personal communication, Mr. M. A. Hannan, Secretary General of the All Pakistan Meat Exporters & Processors Association). With the growing implementation of feed lot fattening as the preferred rearing system and the associated development of industrialized meat production, this loss is continuously decreasing. In addition to these apparent direct costs, indirect costs due to human infections must be considered, albeit data on human cysticercosis in Pakistan are scarce (Singh and Prabhakar, 2002).

As a first step to determine the impact of cysticercosis in the food chain in Pakistan, it was attempted to assess the overall prevalence of cysticercosis in cattle and buffalo population in the Punjab and Sindh region, Pakistan, taking into account animal species, gender and age as possible risk factors.

Materials and Methods

Information about slaughter animals

All cattle and buffaloes slaughtered at a commercial and export-approved abattoir in Lahore, Pakistan, in the period from April to October, 2009, were included in this study. In total, 4836 animals were tested by qualified and trained veterinary meat inspectors. The annual throughput of this slaughterhouse represented roughly 8 % of the entire meat volume from cattle and buffaloes destined for export. Data on age and gender distribution are given in Tables 1a and 1b for cattle and buffaloes, respectively. The animals originated from the Punjab and Sindh regions of Pakistan.

Meat inspection procedure

Every slaughtered animal was examined using extended meat inspection i. e.

- I. conventional examination of cut surfaces of the *Mm. masseteres* (superficial and deep cut) and the pterygoid muscles, the freed tongue, the heart, diaphragm and esophagus (OIE, 2008), the order of examination being depicted in Figure 1,
- II. and, in addition, inspection of three incisions made in the *Musculus triceps brachii* (Ginsberg, 1960; Herenda et al., 2000; Wanzala et al., 2002).

Presumptive cysticerci were identified as spherical objects with 2–20 mm diameter, either containing fluid or caseous masses, or being already calcified (King and Hutchinson, 2007). Identification of *Cysticercus bovis* was based on Murrel et al. (1986) and Abuseir et al. (2006). In detail, fluid-filled, viable cysts were considered mature when they contained a protoscolex, whereas those without a distinct protoscolex were considered immature; degenerating cysts were characterized by a solid or cheesy content.

Statistical processing of results

According to the examination, animals were classified as *Cysticercus* negative, or, if one or more cysts were detected, positive. No distinction was made as regards the number or the viability of cysts detected per animal.

Data obtained via extended meat inspection were analyzed by logistic regression (Statgraphics 3.0; Princeton, NJ, USA) to explore the significance of the following characteristics: species, age and gender on the proportion of infected carcasses (dependent variable), adjusted for the different sample sizes. Efficacy of extended vs. conventional meat inspection was assessed by chi-square-test.

Results and Discussion

Prevalence of *Cysticercus* in cattle and buffalo

Using extended meat examination, cysticerci were detected in 2.1 % of 3731 cattle (Tab. 1a), and in 4.7 % of 1105 inspected buffaloes (Tab. 1b).

Risk factors associated with the presence of cysticerci

According to the information provided upon the delivery of animals at the slaughter house, animals were kept under the free grazing feeding system. In Pakistan, both ruminant

TABLE 1a: *Cysticercus bovis* findings in cattle (*Bos indicus*) examined at Lahore export slaughterhouse, 2009, using extended meat inspection.

Age (years)	Total,	Male,	Female,
	$n_{\text{infected}}/n_{\text{inspected}}$	$n_{\text{infected}}/n_{\text{inspected}}$	$n_{\text{infected}}/n_{\text{inspected}}$
1.5–2.5	38 / 2625	22 / 1841	16 / 784
2.5–4.0	42 / 1106	29 / 770	13 / 336
Total	80 / 3731	51 / 2611	29 / 1120

TABLE 1b: *Cysticercus bovis* findings in buffaloes (*Bubalus bubalus*) examined at Lahore export slaughterhouse, 2009, using extended meat inspection.

Age (years)	Total,	Male,	Female,
	$n_{\text{infected}}/n_{\text{inspected}}$	$n_{\text{infected}}/n_{\text{inspected}}$	$n_{\text{infected}}/n_{\text{inspected}}$
1.5–2.5	18 / 646	11 / 489	7 / 157
2.5–4.0	34 / 459	21 / 284	13 / 175
Total	52 / 1105	32 / 773	20 / 332

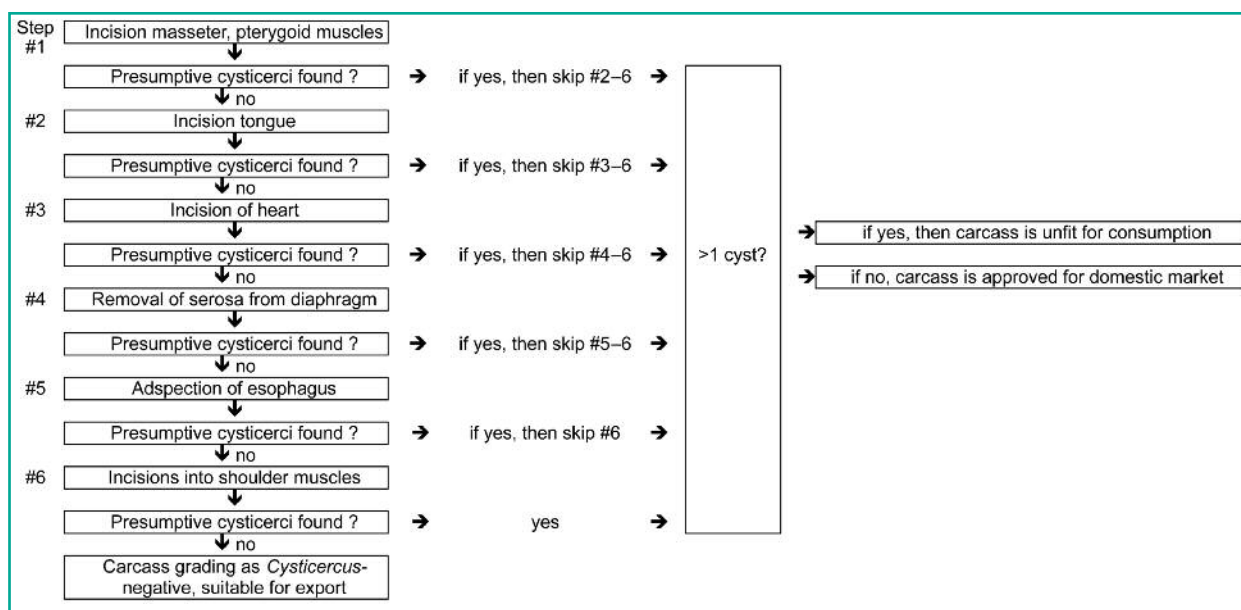


FIGURE 1: Flowchart for inspection for cysticerci in cattle and buffalo carcasses, as performed at Lahore slaughterhouse.

species are usually kept by the nomads in the rural and peri urban areas. Under these conditions, open air defecation (Shikongo-Kuvare, 2007) and probably sewage water (Cabaret et al., 2002) represent a major cause for these animals to have contact with the eggs. In grazing areas e. g. Cholistan and Thar Desert, animals may be infected during rainy seasons as a result of flooding (Allepuz et al., 2009; Kumar and Gebrehiwot, 2011). Also, human feces and dung mixture (which may contain tapeworm eggs) is used as a natural fertilizer on grazing sites, pastures and meadows. In the absence of specific and more detailed information on rearing conditions a risk classification according to holding system was not possible.

The proportion of carcasses found to be infected with cysts was significantly different for species ($P = 0.002$), age ($P < 0.001$) and gender ($P < 0.001$) categories. In detail, the percentage of cysticerci was higher in cattle older than 2.5 years (3.8 %) than in younger animals (1.4 %). For buffaloes, the respective percentages were 7.4 vs. 2.8 % (Tab. 1a, 1b). This was not unexpected, as with increasing age, the animal is obviously more frequently exposed to different sources of infection (Dorny et al., 2000). Also, the occurrence of *Cysticercus bovis* in buffaloes was higher than in cattle. Cysticerci were detected more frequently in female cattle (2.6%) than in male cattle (1.9 %). In buffaloes, cysticerci were less prevalent in male buffaloes (4.1 %) than in female buffaloes (6.0 %). This may be due to the common practice that females are slaughtered at a relatively old age, i.e. after the end of their productive life, whereas males are fattened for a short period on total mixed ration which fulfills all requirements of energy. This type of feeding strategy decreases the likelihood of contracting the infection (Nanda and Toshihiko, 2003).

As the study was only conducted over a 6 months period, we cannot exclude that there might be seasonal differences over a 1 year period. Further studies should consider that issue.

Distribution of cysts

Table 2 indicates that with conventional meat examination, cysticerci were detected in 1.9 % of cattle and 4.3 % of buffalo, whereas with the extended method of meat examination it was 2.1 % and 4.7 % respectively. This increase in detection was, however, not statistically significant ($P = 0.51$). The numbers of carcasses found positive at each examination step (Fig. 1) are given in Table 2.

Significance for public health in Pakistan

Cysticercosis is a public health issue worldwide. The provision of safe food implies that measures need to be taken along the food chain in order to either eliminate this pathogen or to inactivate parasitic stages, as outlined below.

With respect to consumer protection, and under the conditions given in Pakistan, post-mortem examination of carcasses and organs can be considered a cornerstone in public health (FAO, 2008). National legislation gives no clear indication how to examine carcasses for *Cysticercus* and how to decide on the fitness for human consumption. The current practice is, that carcasses with a single cyst detected during extended meat inspection will go on the domestic market. Hence, these carcasses (as well as infected carcasses gone undetected) represent a major hazard for consumers in Pakistan. It is generally assumed that the sensitivity of examination for *Cysticercus* is in the range of 15 to 40 % (Saini et al., 1997; Minozzo et al., 2002; SCVPH, 2000, 2003; Wanzala et al., 2002) which means that most of the infected carcasses would in fact, enter the market. In old animals, most cysticerci (about 78 %; Hathaway and Richards, 1993) are likely to be degenerated. However, according to our study, older bovines (> 2.5 years) account

TABLE 2: Occurrence of *Cysticercus bovis* in different organs of cattle and buffaloes examined at Lahore export slaughterhouse, 2009.

Animal species	Examined carcasses n=	Head muscles n= / %	Tongue n= / %	Heart n= / %	Dia-phragm n= / %	Eso-phagus n= / %	Shoulder muscle* n= / %
Cattle	3731	21/0.6	24/0.6	17/0.4	05/0.13	05/0.13	08/0.2
Buffalo	1105	18/1.6	22/2.0	04/0.4	02/0.20	02/0.20	04/0.4

* this muscle group was additionally examined during extended meat inspection.

ted only for roughly one third of the slaughtered animals. In sum, the uncertainties and ambiguities associated with current practice prompt for additional control measures.

In a number of countries, infected carcasses or the meat cuts thereof would be subject to mandatory deep-freezing or heat treatment, according to well-defined protocols and under supervision of the competent authority (Hilwig et al., 1978; Edwards et al., 1997). In Pakistan, mandatory deep-freezing cannot be implemented for economic reasons (infrastructure as well as energy costs) (Khan and Usman, 2009). Hence, risk measurement largely relies on traditional beef preparation techniques, which effectuate internal beef core temperatures of $> 56^{\circ}\text{C}$ for > 1 second, which is most likely to inactivate the cysts (EC, 2000). However, changes in meat preparation habits (Shehla, 2012) such as consumption of raw or semi-raw products may lead to ingestion of infective cysticerci.

Thus, education of consumers as regards “safe” meat preparation techniques (Kebede et al., 2009) as well as measures in the ante-mortem stage should be considered so as to better control the risk of human taeniosis.

Ante-mortem measures correspond to improvements in biosecurity and would, most probably, also have a reducing effect on other pathogens, thus also improving animal health (Graham et al., 2008).

Conclusions

The data obtained on the presence of cysticerci in slaughter carcasses of cattle and buffalo from the Punjab and Sindh region can serve as a baseline prevalence estimate. There are differences in prevalence according to species, age and gender, but not to an extent which would allow the implementation of risk-based meat inspection. It is not certain that a carcass, in which a single *Cysticercus* has been detected in the predilection sites, will not contain cysticerci in other parts. Thus, the main principle should be to condemn parts infested with cysticercus and to release other parts of the carcass for human consumption mentioning requirements for cooking.

Beef preparation techniques commonly and traditionally applied in Pakistan may be seen as an additional line of defense, but this should be augmented by (1) consumer education, (2) proper labeling of beef from low-infected carcasses mentioning special instructions for cooking, and (3) improvement of the biosecurity in the primary production. For the control of this parasitic disease risk communication from veterinary officials to farmers is essential.

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