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Short Paper

A survey on the bovine amphistomiasis in Mazanderan province, north of Iran

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Summary

This study was carried out to determine the prevalence and intensity of amphistomiasis in native cattle and mixed breed Holstein from Mazanderan province, in the north of Iran in association with sex, age, breed and season. During the 4 seasons of 2008, at meat inspection the rumen and reticulum of 132 native cattle and 104 mixed breed were examined by naked eye for amphistomiasis. All amphistomes found in each cattle were collected separately and preserved in 70% alcohol containing 5% glycerin for further studies. Our findings revealed the overall prevalence rate and Mean ± SE of intensity 33.9% and 864.4 ± 127.2 amphistomes per animal, 40.9% and 1012.5 ± 179.4 in native cattle, and 25% and 557.42 ± 99.9 in mixed breeds, respectively. A few amphistomes were collected from the reticulum of a native cattle. There was no significant relation between the intensity of the infection and the breed (P=0.094), whereas the relation between breed and the prevalence of infection (P=0.0103), and that of age and the infection (P=0.016) were significant. The older group (5≤) harbored more trematodes than ≤2 and 3–4-year-old, and p-values: P=0.026 and P=0.032 were significant, respectively. Difference was observed between the intensity of infection in different age groups. Again a highly significant relation was shown between gender and the prevalence of infection (P<0.001), but not with the intensity of infection (P=0.206). Despite the difference in the seasonal variations of prevalence (P=0.133), and the relation between the intensity of infection and season (P=0.794), these were not statistically significant. At least 20 amphistomes from each infected cattle were stained using aceto-alum carmine and the identified species were: *Calicophoron calicophorn* (42.85%), *Gastrothylax crumenifer* (34.5%), *Paramphistomum gotoi* (21.8%) and *Carmyerius spatiosus* (0.75%). *Calicophoron calicophorn* is a new host and distribution record for studied areas as well as Iran.

Key words: Amphistomiasis, Prevalence, Cattle, Mazanderan province, Northern Iran

Introduction

Paramphistomiasis has been a neglected trematode infectious disease in ruminants, but has recently emerged as an important cause of productivity loss (Anuracpreeda *et al*., 2008). It has a wide geographical distribution in subtropical and tropical areas, where the infection leads to economic losses related to mortality and low productivity (Kilani *et al*., 2003). There is little evidence regarding the pathogenesis of adult flukes to their hosts, but severe damage to the mucosa of the rumen was provoked in heavy infection in experimentally infected sheep (Rolfe *et al*., 1994). A high number of immature worms in the duodenum may affect production, since these parasites causing a lower feed conversion, a loss of weight and/or a decrease in milk production, are responsible for economic losses, morbidity and mortality (Rolfe *et al*., 1991). There is only one report on cattle amphistomes in Iran (Bagheri, 1962) and some others on sheep and goats (Arfaa, 1962; Eslami and Faizy, 1975; Moghadar

and Khanitapeh, 2003). In a more comprehensive study, 10 species of amphistomes were reported from ruminants of Iran (Sey and Eslami, 1981). The present study was carried out to determine the prevalence and intensity of cattle amphistomiasis in Mazanderan province, in the north of Iran in association with age, sex, breed and season.

Materials and Methods

Mazanderan province is located on the southern coast of the Caspian Sea, where enjoys Mediterranean like weather with high humidity and mild temperatures during most months of the year. During 2008, seasonal systematic random sampling was carried out and due to the unavailability of previous reports on the prevalence of infection, the minimum sample size per season was determined to be 33, although for preventing attrition, more samples were taken (41 in autumn, 58 in winter, 64 in spring and 73 in summer). Accordingly, the rumen and reticulum of 132 native cattle and 104 mixed breed Holstein were examined for amphistomes. Sex, age and breed of the examined cattle were recorded and three age groups including: G-1 (≤2 years), G-2 (3-4 years) and G-3 (5≤years) were formed. Amphistomes found in each cattle were counted separately, and 20 were stained using aceto-alum carmine. Their differentiation to the species was carried out according to the morphological characteristics described by Sey (1991).

Statistical analysis

Statistical analysis using Chi-square, the independent samples t-test, one way ANOVA test and post hoc Scheffe were applied on the resulting data to evaluate the relation between prevalence and intensity of cattle amphistomiasis with age, gender, breed and season.

Results

Our findings showed that the overall prevalence rate and Mean ± SE intensity of infection were 33.9% and 864.4 ± 127.2 amphistomes per animal, respectively; while these were 40.9% and 1012.5 ± 179.4 in native cattle and 25% and 557.42 ± 99.9 in mixed breeds. Among the reticulums examined, a few amphistomes were found in one native cattle. The results of prevalence according to breeds, age groups, gender and season are summarized in Tables 1-4.

The identification of stained trematodes to the species revealed 42.85, 34.5, 21.8 and 0.75% *Calicophoron calicophoron*, *Gastrothylax crumenifer*, *Parampistomum gotoi* and *Carmyerius spatiosus*, respectively.

Discussion

Paramphistomiasis has been a neglected trematode infectious disease in ruminants but has recently emerged as an important cause of productivity loss (Anuracpreeda *et al*., 2008). It is still misjudged, as most

Table 1: The prevalence and intensity of amphistomiasis in different breeds

Breed	No. examined	No. infected (percentage)	Mean ± SE intensity	Range
Stray cattle	132	54 (40.9)	1012.15 ± 179.4	2-8000
Mixed breed	104	26 (25.0)	557.42 ± 99.9	32-2000

The independent samples t-test showed no significant relation between the intensity of infection and the breed (P=0.094), but Chi-square showed a significant relation between breed and the prevalence of infection (P=0.0103)

Table 2: The prevalence and intensity of amphistomiasis in different age groups

Age groups (year)	No. examined	No. infected (percentage)	Mean ± SE intensity	Range
≤2	36	9 (25.0)	830.9 ± 257.0	40-2150
3-4	172	55 (32.0)	806.6 ± 160.3	2-8000
≥5	28	16 (57.1)	1081.9 ± 290.6	110-4100

Using Chi-square, significant relation was found between age and the prevalence of infection (P=0.016). Also, One way ANOVA test and post hoc Scheffe showed difference between intensity in different age groups and the older group (5≤) harbored more trematodes than ≤2 and 3-4 year of age, and p-values were (P=0.026) and (P=0.032), respectively

Table 3: The prevalence and intensity of amphistomiasis in different seasons

Season	No. examined	No. infected (percentage)	Mean ± SE intensity	Range
Spring	64	23 (35.9)	783.6 ± 195.8	2-4100
Summer	56	16 (28.6)	760.6 ± 484.2	70-8000
Autumn	58	26 (44.8)	1052.5 ± 184.4	40-3800
Winter	58	15 (25.9)	722.7 ± 290.6	209-2000

Despite difference in the seasonal variations of prevalence, based on Chi-square, no significant seasonality was observed (P=0.133). Also, using One way ANOVA test showed no difference between intensity (P=0.794) and the infection in different seasons

Table 4: The prevalence and intensity of amphistomiasis in different genders

Sex	No. examined	No. infected (percentage)	Mean ± SE intensity	Range
Male	114	26 (22.8)	631.4 ± 121.0	2-2340
Female	122	54 (44.3)	976.5 ± 178.0	32-8000

Again, using Chi-square test a highly significant relation was shown between gender and the prevalence of infection (P<0.001), but not with the intensity using independent samples t-test (P=0.206)

reports on this disease do not quote the responsible species of amphistomes and the various species of the family paramphistomatidae are difficult to identify from a systematic point of view (Mage *et al*., 2002). There is only one report on the presence of *Paramphistomum cervi*, *Cotylophoron cotylophorum* and *Gastrothylax crumenifer* in cattle of Iran (Bagheri, 1962), while in a more comprehensive and morphologically more reliable study (Sey and Eslami, 1981) 10 different species of amphistomes were recorded from ruminants of Iran, of which three species were found in the present study and *C. calicophoron* is a new host and distribution record for studied areas and Iran. On the other hand, regarding the very low prevalence rate of *C. spatiosus* (0.75%) in this survey, it can be assumed that the frequency distribution of other species reported by Sey and Eslami (1981) is limited and depend on the studied regions. Although in Western Europe and Pakistan lesser number e.g. *P. daubneyi*, *P. cervi* and *P. microbothrium* are the most frequent species in cattle, respectively (Mage *et al*., 2002; Javed Khan *et al*., 2006). It is worth mentioning that the pathogenicity of different species of amphistomes is similar to each other (Vercruysse *et al*., 1988). Therefore, mixed infection, which is the rule in Iran, could not produce any complication in the trend of disease production. To compare our results of intensity with other workers according to Macarthur (1994) 20,000 to 25,000 flukes would result in clinical disease, and smaller number would causes significant subclinical diseases in sheep. The mean intensity of infection in both native (1012.15 ± 179.4) and Holstein (557.42 ± 99.9) was light, although 8000 amphistomes were collected from one native cattle. There is little evidence regarding the pathogenicity of adult flukes to their hosts, but severe damage to the mucosa of the rumen is provoked in heavy infection (Rolfe *et al*., 1991). Amphistomiasis is a weather dependent parasitic infection. Mazanderan province, with a moderate temperature and high humidity in most months of the year, enjoys favorable conditions for snail born diseases. Moisture and temperature have a critical role in the propagation of infection, because they affect the hatching of fluke ova, viability of encysted metacercariae and population of snails. Although the infection was found throughout the year, and the least and the most infected seasons were winter (25.9%) and autumn (44.8%), no positive seasonality was observed (P=0.133). These findings are in accordance with Phiri *et al*. (2007) in Zambia, Javed Khan *et al*. (2006) in Punjab Pakistan and Mage *et al*. (2002) in Central France. Amphistomiasis of cattle is largely a disease of young animals, as successive small infections produce an almost complete immunity (Vercruysse *et al*., 1988), but our results showed higher prevalence and intensity in older animals (Table 2), a finding similar to Pfukenyi *et al*. (2005) in cattle of Zimbabwe, but in contrast to Javed Khan *et al*. (2006) in buffaloes of Pakistan and Diaz *et al*. (2006) in cattle of

Spain. Higher percentage and mean intensity in stray cattle, which are free roaming in the environment (40.9%, 1012.15 ± 179.4) in comparison with mixed breed (25.0% and 557.42 ± 99.9) are a natural phenomenon, because they are more commonly in contact with the infected pastures. The results of this study on the prevalence of amphistomiasis in Iran, with 1.5 million mixed and pure breed Holstein, and about seven million native cattle (Anon. 2007), show that amphistomiasis is among the most important parasitic diseases of cattle, at least in regions where climatic conditions are favorable for developing snail born diseases. Therefore, further studies are needed to evaluate the impacts of adult and immature amphistomiasis on animal health as well as animal production.

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