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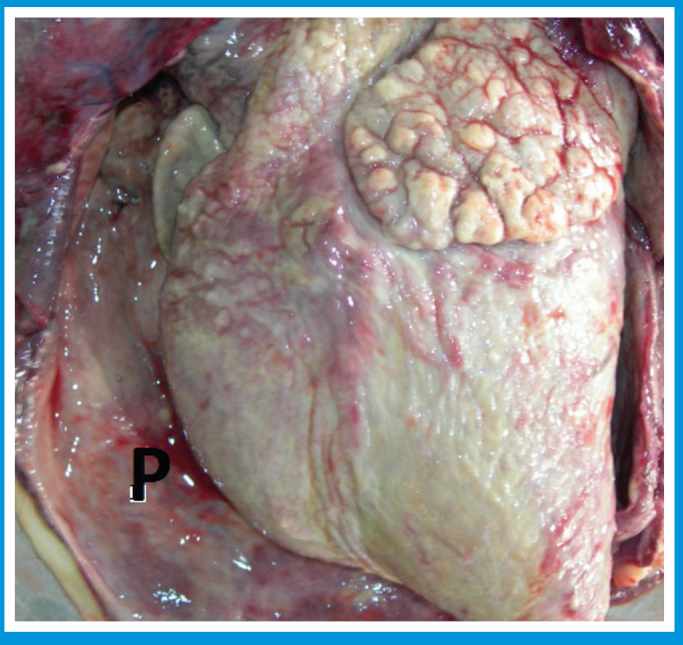

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Original Scientific Article

SEROPREVALENCE OF CATTLE PARATUBERCULOSIS IN SLOVENIA IN 2008 AND A COMPARISON OF DATA FROM CURRENT AND PREVIOUS STUDIES

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Summary: Paratuberculosis is caused by *Mycobacterium avium* subsp. paratuberculosis (Map) and affects many animal species, with ruminants as usual hosts. Due to the lack of successful treatment, infection leads to chronic wasting and death of the affected animals, causing serious economic losses in addition to the spread of public fear connected to the possible role of Map in Crohn's disease. It is also a common disease of ruminants in Slovenia. Because of the lack of prevalence data since 2001, the purpose of this work was to estimate the seroprevalence of paratuberculosis in cattle herds in Slovenia. Animals older than two years in 20% of herds, originating from all different areas of Slovenia were tested in 2008 for the presence of antibodies against Map. A total of 38,374 sera from 6,779 cattle herds were initially examined by the in-house ELISA screening method, and positive or suspect sera were subjected to second screening and final confirmation by applying the Pourquier ELISA Paratuberculosis kit (Institut Pourquier, France). A positive result was obtained in 228 (0.59%) animals from 188 (2.77%) herds, resulting in true-prevalence (TP) estimates of 3.96% at animal and 18.49% at herd level. Currently, TP of paratuberculosis in cattle in Slovenia is lower than at the time of monitoring in 1999, when a comparable number of animals were tested but has remained similar at the herd level. Compared to many European countries, both the animal and the herd prevalences in Slovenia are fairly low, which can be partly attributed to the existence of numerous "family" farms, with a small number of animals per herd, since Map more easily spreads through an infected herd than among different herds. If the breeding strategy changes and animal trade with other countries increases, the present favorable situation in Slovenia will probably also change.

Key words: paratuberculosis; *Mycobacterium avium* subsp. *paratuberculosis;* cattle; seroprevalence; ELISA

Introduction

Paratuberculosis is a chronic infectious disease of ruminants but it also affects many other domestic and wild animals. It is caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map). Map infection is acquired when animals feed on contaminated pastures but the most important mode of transmission is when newborns ingest Map from the faeces of paratuberculous cows; the disease can also be acquired *in utero* and when newborns ingest colostrum or milk from infected cows (1,2). The infection spreads from the intestinal tract to other organs and progresses through three clinical stages: asymptomatic with undetectable Map excretion, asymptomatic but excretory with a gradual increase of Map concentration in the intestinal mucosa and lumen, and symptomatic with heavy shedding of Map (2). The last stage of the disease is characterized by chronic diarrhea and symptoms of generalized infection, such as emaciation, decreased milk production, infertility and death. In cattle herds, animals with the asymptomatic form of paratuberculosis greatly outnumber those with the clinical disease (3,4).

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Fighting paratuberculosis is important in cattle breeding because of the major economic losses it causes (5), in addition to possible transmission of Map to humans in connection with Crohn's disease (6). In the USA, losses are estimated to over 1.5 billion dollars per year (2). Paratuberculosis is also a common disease of ruminants in Slovenia (7). The first case was detected in 1961 in imported Jersey cows. No other cases were reported until 1993, when paratuberculosis was found in a sheep flock; since then, several outbreaks of the disease in cattle, goats and sheep have been documented (8).

An estimation of prevalence is needed for taking the right measures to control the disease. Systematic screening of paratuberculosis in Slovenia, based on the complement fixation test, therefore began in 1995. In order to estimate the prevalence and geographic distribution of paratuberculosis in Slovenia, 20% and 5% of cattle in all herds were tested in 1995 and 1996, respectively (7). In the following two years, 3% and 5% of cattle in all herds were tested by ELISA, which became the most commonly used assay for Map antibody testing (7). In 1999, 10-15% of cows and pregnant heifers were tested, while in 2000 and 2001, the survey was limited to bulls' mothers herds because of their significant involvement in selection for reproduction (9).

Since 2001, no data on paratuberculosis prevalence in Slovenia have been available. The purpose of this study, therefore, was to estimate the current seroprevalence of paratuberculosis in cattle herds in Slovenia and to compare data from current and previous studies.

Materials and methods

Animals older than two years were tested in 2008 for the presence of antibodies against Map in 20% of cattle herds, randomly selected from all areas of Slovenia (Table 1). A total of 38,374 sera from 6,779 herds were tested. After the initial lower-cost screening, performed by in-house ELISA (10), with similar sensitivity but lower specificity as demonstrated in a preliminary study comparing the in-house and the subsequently employed commercial ELISA kit, all positive or suspect sera were subjected to additional screening using the Pourquier ELISA Paratuberculosis – Paratub.Serum-S kit (Institut Pourquier, France) according to the manufacturer's instructions. Confirmation of positive samples was performed by using the Pourquier ELISA Paratuberculosis – Paratub.Serum-B antibody verification kit by the same manufacturer. Only animals testing positive using the verification kit and herds with at least one test-positive animal were considered Map-seropositive.

In addition to estimating the current seroprevalence of paratuberculosis in Slovenian cattle, a comparison of the prevalence data from the present study with data collected in previous studies was made (Table 2). The apparent prevalence (AP) was calculated as defined by Nielsen and Toft (11). The true prevalence (TP) was estimated from the AP by correction with the Rogan-Gladen estimator (12) according to the following formula: $TP = (AP + Sp - 1) \times (Sp + Se - 1)^{-1}$; the previously reported most likely test accuracies for cattle serum ELISA by Idexx Laboratories Inc. (USA) and Institut Pourquier (France) were employed: a sensitivity (Se) of 0.088 and 0.15, and specificity (Sp) of 0.976 and 1.0, respectively (11). At the herd level, the parameters AP, TP, Sp and Se were termed HAP, HTP, HSp and HSe; animal- and herd-level parameters were considered similar (11).

Results

A total of 228 (0.59%) animals from 188 (2.77%) herds tested positive for paratuberculosis (Table 1), resulting in calculated true-prevalence estimates of 3.96% (TP) and 18.49% (HTP) (Table 2). The majority of the positive herds originated from areas of NM and MB located in the south-eastern and north-eastern parts of Slovenia, respectively. In herds with more than one seropositive animal, almost half (47%) of the positive animals were of the Black-and-White (Holstein-Friesian) breed (*data not shown*).

Comparison of data from the present and previous studies on paratuberculosis seroprevalence in cattle in Slovenia is shown in Table 2. The estimates of TP at animal and herd level were highest in 1999 (TP 15.50%) and 1998 (HTP 63.74%) respectively. In 2000-2001, HTP was also high (77.26%) but reflected prevalence in bulls' mothers herds only. In view of the comparable number of cattle sampled in 1999 and 2008, albeit originating from a different number of herds, estimates of TP from these two periods were compared: in 2008, the prevalence of paratuberculosis at the animal level decreased (15.50% in 1999 vs. 3.96% in 2008) but remained similar at the herd level (25.69% in 1999 vs. 18.49% in 2008).

Table 1: Distribution of collected and positive samples and number of investigated and infected herds according to different areas of Slovenia

Area*	No. of samples		No. of herds	
	Collected	Positive (%)	Investigated	Infected (%)
LJ-CE	12692	71 (0.56)	2242	64 (2.85)
MB	6470	37 (0.57)	1143	35 (3.06)
PT	2900	8 (0.28)	512	8 (1.56)
KR	3712	28 (0.75)	656	17 (2.59)
NM	5934	53 (0.89)	1048	38 (3.63)
MS	3684	19 (0.52)	651	17 (2.61)
GO	2982	12 (0.40)	527	9 (1.71)
Total	*38374*	*228 (0.59)*	*6779*	*188 (2.77)*

* LJ-CE, area of Ljubljana and Celje; MB, area of Maribor; PT, area of Ptuj; KR, area of Kranj; NM, area of Novo mesto; MS, area of Murska Sobota; GO, area of Nova Gorica

Table 2: Animal and herd level apparent and true prevalence of paratuberculosis in cattle in Slovenia obtained from the present and previous studies

Study period	Animals				Herds				Test	Ref.
	All[a]	Pos[b]	AP[c] [%]	TP[d] [%]	All[a]	Pos[b]	HAP[c] [%]	HTP[d] [%]		
1997	11513	47	0.41	-31.12	1690	48	2.84	6.89	A	7
1998	12082	140	1.16	-19.39	2423	157	6.48	63.74	A	7
1999	38469	1305	3.39	15.50	26088	1055	4.04	25.69	A	9
2000-2001	9388	41	0.44	2.91	302	35	11.59	77.26	B	9
2008	38374	228	0.59	3.96	6779	188	2.77	18.49	B	This study

Note: The study period 2000-2001 contained cattle sera from bulls' mothers herds only. The employed paratuberculosis ELISA kits for cattle sera were manufactured by Idexx Laboratories Inc., USA (A) and Institut Pourquier, France (B).

[a] No. of collected samples (animals) and investigated herds (herds); [b] No. of positive samples (animals) and infected herds (herds); [c] Apparent seroprevalence of paratuberculosis at animal (AP) and herd (HAP) levels according to Nielsen and Toft (11); [d] True seroprevalence of paratuberculosis at animal (TP) and herd (HTP) levels according to Nielsen and Toft (11); negative TP should be considered as 0%

Discussion

The slow progression of paratuberculosis, the non-specific clinical signs, irregular faecal shedding of Map, the long incubation period of the disease and slow growth of Map on culture media make reliable diagnosis a difficult task, especially due to the lack of highly sensitive and specific diagnostic tests (2,13). Enzyme immunoassays are very suitable for the detection of Map antibodies but have to be adapted to remove cross-reacting antibodies (14). However, the faecal shedding of large quantities of Map provides a pressing reason for early and reliable diagnosis in order to limit the spread of paratuberculosis within and among cattle herds. To control the spread of the disease, farms rearing animals for reproduction must be free of paratuberculosis or, under Slovenian national legislation, they lose their status of a farm with a permit for ova and embryo donation (9). In addition, an annual governmental decree on the general monitoring of paratuberculosis in Slovenia was passed from

1995 to 1999, but has not been renewed since then. Due to the lack of financial support, data on the seroprevalence of paratuberculosis in cattle herds in Slovenia has been lacking for the past several years.

The present study showed that the herd prevalence in Slovenia has remained at almost the same level as it was about ten years ago and it is fairly low compared to many European countries (11). This can be partly attributed to the small number of animals per herd, *i.e.*, family-farm breeding, which results in the limited spread of Map between different herds (9). To some extent, differences in prevalence on both animal and herd levels observed over the years in Slovenia, reflect not only the actual prevalence but also the different populations tested, the number of animals and herds included in the test and the use of ELISA kits with different sensitivities. However, comparing data from the present study with previous data leads to interesting conclusions. For example, a marked increase in prevalence at the herd level was observed for the period from 1997 to 1998 (7). In those two years, mostly older animals were selected for testing, since paratuberculosis has slow progression and ELISA tests will usually not detect infected animals aged less than two years (15). In 1998, a larger number of herds were selected for monitoring in comparison to 1997 and more older animals were tested. A higher HTP was therefore not surprising, and the extent of the increase may also be partially explained by the improvement of the Idexx ELISA test reported by the manufacturer for 1998. In 1999, a marked increase in prevalence at the animal level was observed (9). More animals were tested in comparison to 1998, leading to a higher TP estimate. In contrast, HTP in 1999 decreased, possibly because many more cattle herds were inspected, with an average of 1.47 animals per herd selected for testing; with fewer animals tested per herd, the probability of detecting Map-positive herds was lower. Due to a change in the selection criteria for animals and herds (*i.e.*, all animals over the age of two years from bulls' mothers herds) and in the ELISA kit for testing (9), the results from the period 2000-2001 are difficult to compare with the previous ones; since 2000, the ELISA kit manufactured by Institut Pourquier (France), with higher specificity and sensitivity (11), has been used for Map-serological testing in Slovenia.

In 2008, all animals older than two years were tested, originating from 20% of randomly selected cattle herds in Slovenia. These data were compared to those from 1999, as reflecting sampling groups of a similar size, although a lower number of herds were inspected in 2008, with a higher number of tested animals per herd (an average of 5.67 animals per herd in 2008 vs. 1.47 in 1999). Since the disease is less prone to spread between herds than within an infected herd, the lower number of inspected herds does not necessarily mean a markedly lower number of Map-positive animals, if more animals per herd are sampled. Due to the non-homogeneous distribution of Map-positive cattle herds in Slovenia, the random-sampling strategy was of utmost importance for generating reliable data on the current seroprevalence of paratuberculosis in cattle. Since the ELISA kit for Map-serological testing in 2008 had higher sensitivity and specificity than the kit employed in 1999 (11), the lower TP at the animal level probably reflects a more favourable present situation in Slovenia.

In many countries, herd level prevalences are likely to be >50% and estimates of animal level prevalences have been reported to be approximately 20% or at least 3-5% in several countries (11). Despite changes in the criteria for the selection of animals in Map-testing during previous years, Slovenia still ranks among countries with the lowest paratuberculosis prevalence at the animal and herd levels. The relatively good present situation could change rapidly in the near future, due to the unlimited trade of animals in the European Union. Moreover, in-country animal trade, including large dairy cattle herds with the Black-and-White breed, which currently represents 19% of cattle in Slovenia and is most commonly infected, may also contribute to the spread of paratuberculosis.

In general, our findings contribute to current knowledge on paratuberculosis prevalence in the European countries. Due to the increasing trade and changes in the animal breeding strategy, *i.e.*, reduction in the number of herds and increase in the number of animals per herd, the promising results for our country are an encouragement to policy makers to make a prompt decision and prepare effective measures for surveillance and control of the disease.

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SEROPREVALENCA GOVEJE PARATUBERKULOZE V SLOVENIJI V LETU 2008 TER PRIMERJAVA S PODATKI PREDHODNIH ŠTUDIJ

D. Kušar, M. Ocepek, K. Logar, M. Pate, B. Krt

Povzetek: Paratuberkulozo povzroča bakterija *Mycobacterium avium* subsp. paratuberculosis (Map) in prizadene mnoge živalske vrste, njeni običajni gostitelji pa so prežvekovalci. Ker uspešno zdravljenje ne obstaja, okužba vodi do kroničnega hiranja in pogina prizadetih živali. To povzroča znatne ekonomske izgube, poleg tega pa tudi širi strah, ki je povezan z možno vlogo Map pri kronovi bolezni (Chronova bolezen). Paratuberkuloza je pogosta bolezen prežvekovalcev tudi v Sloveniji. Zaradi pomanjkanja podatkov o prevalenci od leta 2001 naprej je bil namen našega dela oceniti seroprevalenco paratuberkuloze v govejih čredah v Sloveniji. Leta 2008 smo v 20 % čred z vseh področij Slovenije živali, starejše od dveh let, testirali na prisotnost protiteles proti Map. Z domačim ELISA presejalnim testom smo na začetku raziskave pregledali 38374 serumov živali iz 6779 govejih čred. Vse pozitivne ali sumljive serume smo pregledali z drugim presejalnim ter nato s končnim potrditvenim testom Pourquier ELISA Paratuberculosis kit proizvajalca Institut Pourquier, Francija. Pozitivnih je bilo 228 (0,59 %) živali iz 188 (2,77 %) čred, kar smo lahko ocenili kot 3,96 % in 18,49 % pravo seroprevalenco (PS) na nivoju živali in čred. Trenutno je PS paratuberkuloze goved v Sloveniji nižja kot leta 1999, ko smo testirali primerljivo število živali, vendar je ostala podobna na nivoju čred. V primerjavi z mnogimi evropskimi državami je prevalenca v Sloveniji tako na nivoju živali kot na nivoju čred precej nizka. To zaradi lažjega širjenja Map znotraj okužene črede v primerjavi s širjenjem med različnimi čredami lahko delno pripišemo dejstvu, da je v Sloveniji mnogo »družinskih« kmetij z majhnim številom živali v čredi. Če se bo način reje spremenil in se bo med državami povečalo trgovanje z živalmi, se bo verjetno spremenila tudi trenutno ugodna situacija v Sloveniji.

Ključne besede: paratuberkuloza; *Mycobacterium avium* subsp. paratuberculosis; govedo; seroprevalenca; ELISA

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Case Report

TRAUMATIC RETICULO-PERICARDITIS (TRP) IN SHEEP: A REPORT OF 4 CASES IN A HERD

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Summary: Traumatic reticulo-pericarditis (TRP) was diagnosed in four sheep (one ram and three ewes) during postmortem examination. Gross pathology revealed the penetration of a large needle and three electrical wires through reticular wall into the pericardial sacs, regional artery and myocardium. The prominent findings in the ram were acute cardiac tamponade and hemothorax, whereas pyothorax, enlargement and thickening of the pericardium and peritonitis were the prominent findings in ewes. Disseminated abscesses in spleens, lungs, and livers were also found. During microscopic analysis, fibrosis and inflammation with neutrophilic and lymphocytic infiltrate were observed in the pericardium, epicardium and myocardium. *Arcanobacterium pyogenes* was isolated from fibrinopurulent exudates and disseminated abscesses of the affected organs.

Key words: traumatic reticuloperitonitis-pericarditis; sheep; cardiac tamponade; hemothorax; pyothorax

Introduction

The occurrence of Traumatic reticuloperitonitis-pericarditis (TRP) can be expected in ruminants (1, 2, 3, 4). TRP occurs after penetration of the reticular wall, diaphragm, pericardial sac, and myocardium by sharp metal objects (2, 3, 5, 6). While TRP has been frequently reported in the mature dairy cattle, it occasionally occurs in beef cattle and rarely in sheep and goats (4, 7, 8). Although, many veterinarians assume that the occurrence of Traumatic reticulo-pericarditis is rare in small ruminants, we describe four occurrences in a sheep herd in the present report.

Case history

Within a six-month period, four sheep from one herd including one ram (case 1) and three ewes (cases 2, 3 and 4) died and were referred to the Department of Pathology of Faculty of Veterinary Medicine, University of Tehran, Iran for necropsy. The herd consisted of 350 Moghan ecotype sheep including 210 ewes, 105 lambs, and 35 rams. This sheep ecotype is raised for milk, meat and wool production in Iran. The farm from where the herd originate is situated in the suburb of Tehran, Iran. The herd grazed in a pasture and had access to the salt mixtures ad libitum. They had access to the water from a nearby stream. All sheep were maintained on the pasture during the day and housed at night. The sheep were routinely dewormed with albendazole and vaccinated against brucellosis, anthrax, sheep pox, and FMD based on the program instructed by Iranian Veterinary Organization. The history of the cases according to the owner declaration was as follows:

Case 1: A four-year-old ram, weighing 50 kg, with a history of four days of anorexia, reluctance to move and rise, coughing, decreased fecal production with severe abdominal distention and finally a sudden death.

Case 2: A five-year-old, non-pregnant ewe, weighing 35 kg, with a fifteen-day history of anorexia, losing weight and weakness, arch back, respiratory discomfort, reluctance to move and mild abdominal distention.

Case 3: A three-year-old, non-pregnant ewe, weighing of 25 kg, with six days history of anorexia,

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losing weight and weakness, bruxism and reluctance to move.

Case 4: A four-year-old ewe, weighing 30 kg, showing symptoms of anorexia, losing weight and weakness, reluctance to move, respiratory discomfort, and frequently putting its left forelimb on the crib, during nine days after lambing.

Results

Necropsy findings

Case 1: The exploration of the ruminal and abomasal contents revealed accumulated phytobezoars. Locally extensive fibrinous adhesions between the reticulum and diaphragm were found. The ram had a large needle (9 cm) that penetrated through the reticular wall into the pericardium, regional arteries and left ventricle causing pericarditis, myocarditis, acute cardiac tamponade, and simultaneous hemothorax (Figures 1 and 2).

Case 2: The Gross pathology revealed the penetration of an electrical wire (7 cm) from the reticular wall into the pericardial sac. In the thoracic cavity, a large quantity (300 ml) of turbid, foul-smelling fluid containing clots of fibrin were observed (Figure 3). The pericardial sac was greatly thickened and fused to the pericardium by a fibrinous connective tissue. The reticulum, diaphragm and peritoneum contained numerous fibrous adhesions. Disseminated abscesses in the spleen, lungs, and liver were also seen.

Case 3: There were four electrical wires (2.5, 3, 4.5, and 6 cm) in the ruminal contents. Signs of a wire perforation into the cranioventral aspect of the reticulum were observed. The perforation site was surrounded by inflammation and hemorrhages (Figure 4). The pericardial sac was enlarged and discolored by the fibrinopurulent exudate. Local fibrinous peritonitis and disseminated abscesses in the spleen, lungs, and liver were also evident.

Case 4: Two electrical wires (3 and 5.5 cm) were found in the rumen and one (6. 5 cm) was embedded in the reticular wall and penetrated into the pericardial sac. The pericardial sac was notably thickened and fused to the pericardium by a fibrinous connective tissue (Figure 5). The thoracic cavity was filled by 250 ml of the foul-smelling, dirty yellowish fluid. There were small adhesions between the serosal surface of the abomasum and parietal peritoneum. Like previous cases, disseminated abscesses in the spleen, lungs, and liver were also observed.

Histopathological examination

Similar findings were found in all four cases, except for the identification of the *sarcocystis oocysts* in case 2. They included fibrosis and inflammation with neutrophilic and lymphocytic infiltrate in the pericardium, epicardium and myocardium as well as pulmonary edema and congestion. Microscopic observations of the livers and lungs revealed variable sized abscesses surrounded by proliferating fibroblasts and connective tissue.

Bacteriological culture

Arcanobacterium pyogenes was isolated from the fibrinopurulent exudates of the thoracic cavities and disseminated abscesses of spleens, lungs, and livers.

Diagnosis

These findings strongly support the diagnosis of TRP. Accordingly, death most likely occurred due to acute cardiac tamponade in the ram and due to chronic heart failure (CHF), pleuritis and peritonitis in the ewes.

Pasture investigations

In an attempt to identify the cause of the disease, we investigated the pasture. The presence of several factories close to the pasture which are producing different types of medical and industrial instru-

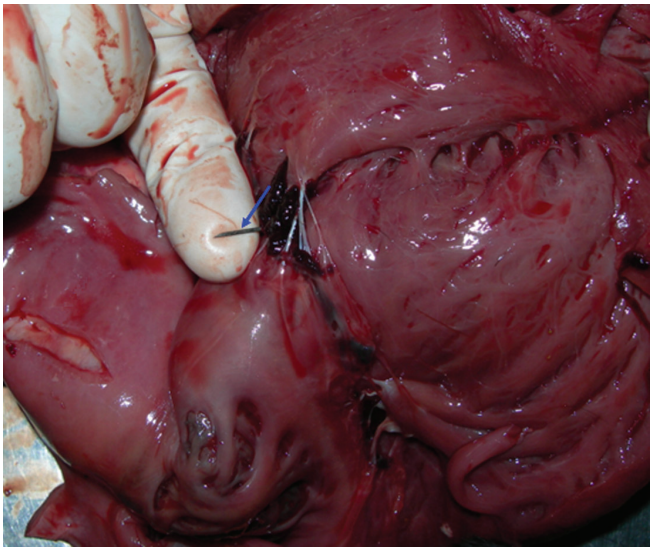

Figure 1: Sharp needle penetrating through the myocardium of the left ventricle (arrows)

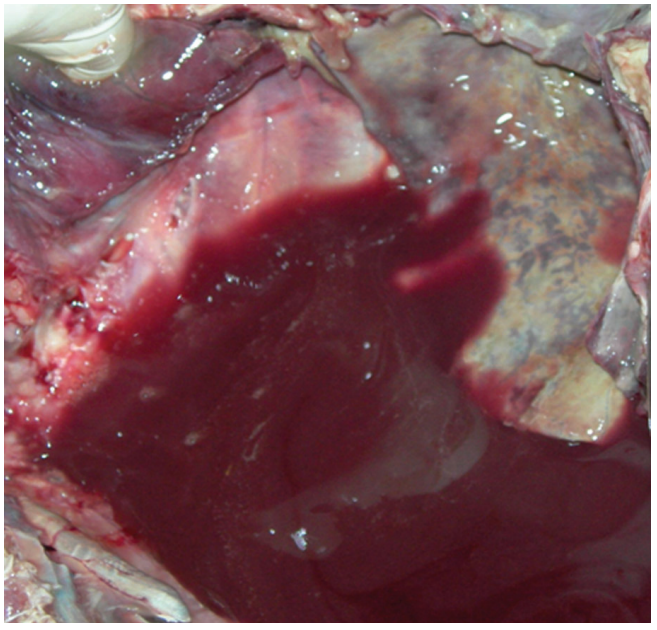

Figure 2: Thoracic cavity filled with blood (hemothorax)

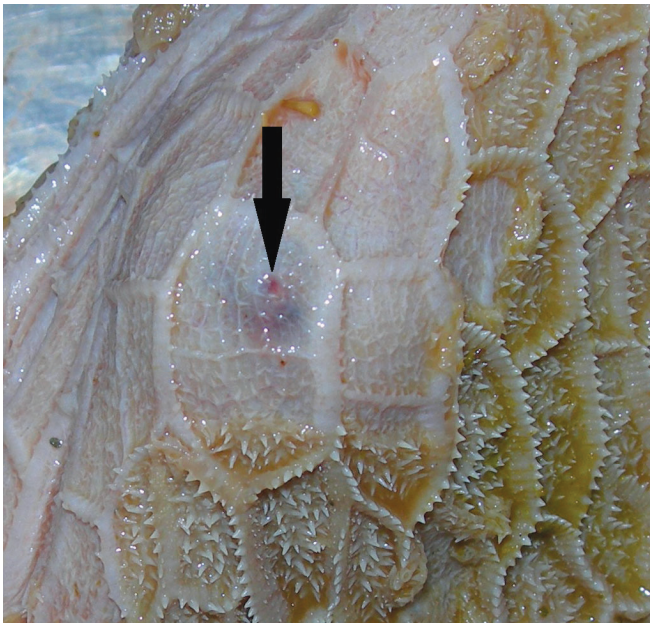

Figure 4: A wire perforation sign into the reticulum of the ewe was presented by the surrounding inflammation (arrow)

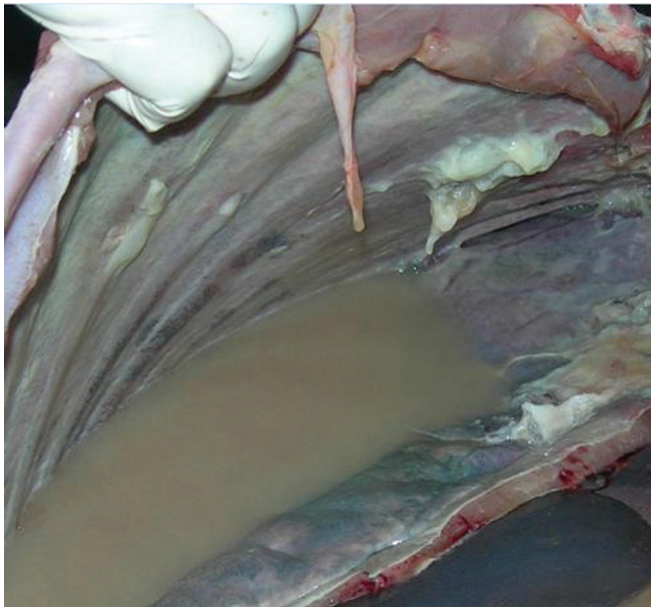

Figure 3: Pleural cavity filled with foul-smelling fluid and clots of fibrin (pyothorax) from one of the ewes

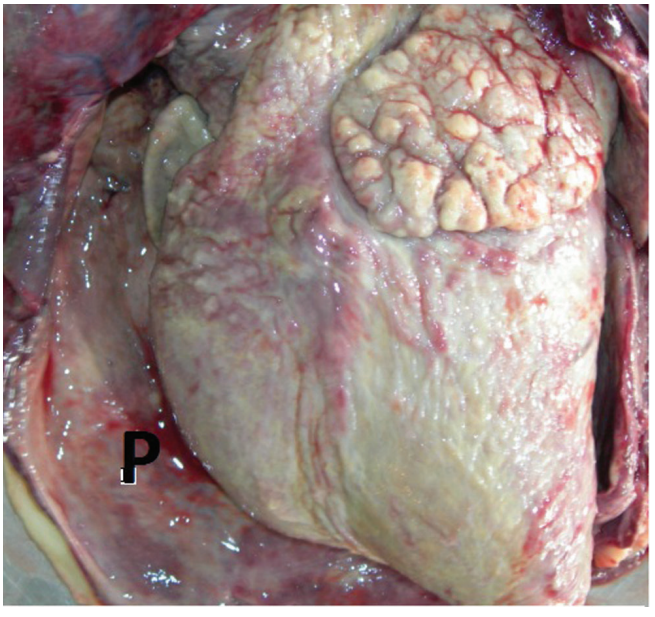

Figure 5: Fibrinopurulent pericarditis due to the traumatic reticulopericarditis; P: pericardium

ments such as syringes, needles and electrical wires, were noted. The results of our investigations showed that these factories have a considerable impact on the grazing pasture pollution by the metal objects.

Discussion

The increase in the intra-abdominal pressure due to the pregnancy and ruminal tympany may facilitate the penetration of the foreign bodies into the reticular wall and other abdominal and thoracic organs in ruminants (1, 9, 10). Furthermore, alimentary tract obstruction and abdominal distention due to phytobezoars have also been described in ram, buffalo, cattle and giraffe (11, 12, 13, 14). In our cases, it seems that increase in the intra-abdominal pressure during pregnancy and subsequent lambing as well as alimentary tract obstruction by the

phytobezoars, might facilitated the penetration of the metallic objects through the reticular walls into the abdominal and thoracic organs. The penetration of the wall of the reticulum by a sharp foreign body may produce different types of peritonitis or may proceed beyond the peritoneum and cause damage of other organs and consequently pericarditis, cardiac tamponade, pneumonia, pleurisy and hepatic, splenic, pulmonary, or diaphragmatic abscesses (2, 3, 6). In suppurative pericarditis, the pericardial surface is notably thickened by white, often rough, shaggy appearing masses of fibrous connective tissue. Fibrous adhesions between the external and internal surfaces of the viscera and pericardium can be observed in chronic inflammatory lesions (15). Similar to these signs, an acute cardiac tamponade and hemothorax were observed in the ram in our study. These defects developed as a consequence of a large needle penetration through the reticular wall into the left ventricle and regional arteries, what caused sudden cardiac death. In the ewes, a similar phenomenon is believed to be the cause of the pericarditis, myocarditis, peritonitis, pleuritis and finally death. The macroscopic and microscopic findings such as pericarditis, myocarditis, pulmonary edema and congestion, pyothorax, peritonitis, and disseminated abscesses in the spleens, lungs and livers are in agreement with the literatures (1, 6, 7, 8).

Sarcocystosis is commonly found in slaughtered sheep in Iran and a high prevalence of sarcocystosis (33.93%) was reported by *Daryani et al* (16). The possibility of the involvement of *sarcocystosis* in heart failure is miniscule because it usually presents primarily as a neurological disorder in sheep (17, 18). In case 2, however, *sarcocystosis* could contributed to the diminished cardiac performance and deterioration of clinical sings, although the relative weakness of the infestation makes this possibility unlikely.

During microbiological analyses, *Archanobacterium Pyogenes* was isolated from the fibrinopurulent exudates and disseminated abscesses of the affected organs. In accord with our findings, Tadayon et al. in 1980 reported that *Arcanobacterim* Spp. is a common finding in cultures of abscesses of sheep (19).

There is strong evidence that sheep are selective feeders and ingest significantly fewer foreign bodies in comparison to the cattle (1, 6, 20). In this respect, while the incidence of foreign body lesions in the cattle has been reported to be 7 % to 21 %, incidence rate of foreign body lesions in sheep and lambs was reported to be much lower, between 1 % and 2 % in different studies (4, 10, 21, 22). Therefore, TRP would be rarely expected in the sheep, goats, and lambs (1, 4, and 6). In the present report, it was found that the incidence of TRP in examined herd is substantially higher in comparison to the other reports. This is most likely due to the serious pollution of the grazing pasture by the metal objects from the nearby industrial objects. The same observations have been reported in sheep in Jordan as the results of heavy environmental pollution (7, 23). As a preventive approach, it was suggested to the owners that the herd should not be grazing in the polluted area, and consequently, there were no new cases of TRP in the follow up during subsequent 4 months. Therefore, our study suggests that TRP may play an important role in animal deaths also in sheep, especially when animals are grazing on a polluted land.

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TRAUMATSKI RETIKULOPERIKARDITIS (TRP) PRI OVCI: PRIMER ŠTUDIJE OVC V ČREDI

E. Torki, M.R. Mokhber Dezfoli, F. Sasani, F. Baghban, M. Shahabi, M. Motaghinejad

Povzetek: V prispevku je predstavljen primer travmatskega retikuloperikarditisa (TRP) pri štirih ovcah (enem ovnu in treh ovcah). Opisano je prodiranje velike igle in treh električnih žic skozi steno kapice v osrčnik in srčno mišičnino. Z raztelesbo smo pri ovnu ogotovili vnetje srčne mišice, srčno temponado in nabiranje krvi v prsnem košu, pri ovcah pa zadebelitev osrčnika in posrčnice ter vnetje srčne mišice. Poleg tega smo pri ovcah opazili še piotoraks ter razsejane ognojke po pljučih in jetrih. patohistološko preiskavo smo v vseh primerih ugotovili fibrozo osrčnika, posrčnice, endokarda in srčne mišice ter vnetje z infiltrati nvetrofilnih granulocitov in limfocitov. V rezinah jeter in pljuč so bili ognojki različnih velikosti, obdani s fibroblasti in vezivom. Iz fibrinsko-purulentnega izcedka in razsejanih ognojkov iz patološko spremenjenih organov je bila izolirana *Arcanobacterium pyogenes.*

Ključne besede: travmatski retikuloperikarditis; ovca; hemotoraks; piotoraks

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Case Report

EFFECTIVE TREATMENT OF GIARDIOSIS IN PIGS BY ALBENDAZOLE

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Summary: Giardiosis is a parasitic disease prevalent worldwide that affects humans, domestic and wild animals and can be responsible for chronic diarrhoea. Diarrhoea started in the time of wintering at the stables of the Institute for the Health Care of Pigs. Two sows (Krsko polje breed), and four piglets aged two months (the Krsko polje breed) were affected. Rectal smears were sent for bacteriological examination. Faecal samples were examined by microscopy using the SAF method for concentrating protozoa and by direct immunofluorescence (DIF).

No pathogenic bacteria were isolated at the first bacteriological examination but the second examination confirmed the presence *of Campylobacter coli. Giardia* sp. was detected by SAF and DIF at the first test. The pigs were treated with Monil®5% (Pliva d.d., Croatia) with 20 mg/kg albendazole, *per os*, once daily, for 3 days.

After 3 days therapy the diarrhoea stopped and the pigs were clinically healthy and remained without diarrhoea for the next four months. Seven days, ten days and eleven days after the treatment all tested samples were negative by SAF and DIF.

Key words: pig; Giardia; treatment; albendazole

Introduction

Giardiosis is a worldwide parasitic disease that affects humans, domestic and wild animals (1) and can be responsible for chronic diarrhoea (2, 3). *Giardia* is a protozoan, which is bilaterally symmetrical, with eight flagella, and has a large adhesive disk on the body's ventral surface (trophozoit). The disk enables attachment to the epithelial cells of the intestinal mucosa. The simple life cycle of *Giardia*, involving an environmentally resistant cyst, provides many opportunities for the parasite to be transmitted directly from one infected individual to another, or indirectly through contamination of the environment or water and food (4). The cysts are more common in pens with solid floors, and piglets and weaners appear to be infected by the sows (5). The parasite colonizes the small intestine of animals and may lead to moderate to severe diarrhoea (6) or clinical signs may be absent (5). *Giardia* infections have been reported in pigs in all groups from nursing piglets to boars and sows from Europe, Australia, Asia and North America with prevalence ranging between 0,1% and 20%, but usually not associated with clinical illness (7, 8, 9, 10). *Giardia* cysts were identified in 3.8% of piglets, 9.8% of weaners, 10.8% of growers, 15% of finishers, 5.7% of boars and 4.1% of sows (6). Diagnosis of Giardia by traditional microscopic methods following the application of faecal concentration techniques, especially zinc sulphate flotation and centrifugation (11) which remains a reliable indicator of infection. However, the detection of *Giardia* by microscopy or faecal ELISA is of limited epidemiological value, especially in terms of the source of infection. The development of direct immunofluorescence microscopy has generally improved the sensitivity of detecting and quantitating faecal *Giardia* cysts and may allow for more accurate determination of prevalence rates and cyst excretion intensities than conventional microscopy (12). With Giardia,

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molecular techniques, particularly PCR-based procedures are more sensitive and specific than 'conventional' diagnostics that rely on microscopy and/or immunodiagnostics (13). Infections with Giardia stimulate humoral immunity that results in self-limiting infection in many animal species (6). Unfortunately, it may take several months for the host to produce protective antibodies that can eliminate the parasite. Chemotherapy may be highly effective in eliminating infection (4). Two benzimidazole drugs, albendazole and mebendazole, have been suggested to exhibit clinical efficacy against human giardiosis (14, 15, 16, 17). Although the claims for mebendazole were disputed (18,19, 20), in vitro studies have shown that albendazole, mebendazole and fenbendazole are much more effective against *Giardia* trophozoites than metronidazole, tinidazole, or quinacrin (21, 22, 23, 24, 25). Benzimidazoles are well known as inhibitors of the polymerization of tubulin to microtubules. Because microtubules are major components of the four pairs of flagella, the median body, and the ventral disk of *Giardia* trophozoites, it is likely that these drugs exert their activities against *Giardia* through the inhibition of their attachment to the intestinal mucosa (24, 26, 25).

In this report, the efficacy of albendazole against *Giardia* infection of pigs is presented.

Materials and methods

Pigs

Two sows of the Krsko polje breed, two years old, one pregnant, were taken to the stables of the Institute for health care of pigs for wintering from the Centre for sustainable recultivation, Vremščica where pigs are kept outdoors.

The Krsko polje or "black belted" pig is the only Slovenian autochthonous breed of pig. It is an extensive breed, whose characteristics are resistance, good adaptability to poor rearing and feeding conditions, and excellent meat quality. These pigs have relatively large litters but too many stillborns and additional losses before the piglets are weaned (27).

Pigs, housing

The non-pregnant sow was housed in a box, size 1.2 x 4.2m with concrete floor. The pregnant sow was housed in the farrowing box size 1.3 x 3m, with a concrete floor. The sow farrowed four piglets 14 days after transportation. The piglets were not weaned during the wintering. The temperature in the pen was between 16°C and 19°C, the farrowing box also had a heating lamp. Boxes were cleaned twice daily when pigs were fed. The piglets were fed with Pu-starter (Jata Emona, Ljubljana, Slovenia) ad libitum and they suckled. Sows were feed with S-doj (Jata Emona, Ljubljana, Slovenia) ad libitum. Water access from public water supply was ad libitum on water nipples in both boxes.

The pigs were reared according to the Council directive for minimum standards for the protection of pigs (2008/120/EC).

Clinical signs

While the sows were stabled they were examined clinically (rectal temperature, respiratory rate, faeces consistency). All tested parameters were in normal ranges. At the age of two months, diarrhoea was noted in all four piglets and also in the two sows. The diarrhoea was grey-green or grey-yellow in colour, and the backsides of pigs were smeared.

Collection of samples

Rectal smears and faecal samples were taken from both sows and piglets at the beginning of the diarrhoea, and 7, 10, and 11 days following the treatment. Rectal smears were sent to the laboratory for bacteriological examination. Faecal samples were sent for parasitological examination.

Bacteriological examination

Samples were inoculated on nutrient agar (Oxoid) supplemented with 5% of sheep blood and Drigalski agar (Oxoid) and incubated at 37°C for 24 hours.

For detection of *Salmonella*, samples were enriched in buffered peptone water (Biolife) at 37° C for 18 h and Rappaport Vassiliadis broth (Merck) at 41,5° C for 24 h, then subcultured onto solid selective media XLD agar (Biolife) and Rambach agar (Merck). Both were incubated at 37° C for 24 h.

Parasitological examination

SAF method for concentrating protozoa

2 to 5 g of faeces was diluted with 10 ml SAF (Sodium acetate 1.5 g, acetic acid, glacial 2.0 ml,

formaldehyde, 37 to 40% solution 4.0 ml, distilled water 92.0 ml). After homogenisation, the mixture was stood for 30 minutes, then passed through a filter (gauze) and centrifuged for 1 minute at 2000 rpm. The supernatant was removed, leaving 1 ml of sediment. 7 ml of physiological solution (9.0 g NaCl in 1000 ml solution) and 2 ml of ether were added to the sediment and centrifuged 3 minute at 2000 rpm. The supernatant was removed and a few drops from the 1 ml of sediment used for microscopic observation at 400-x magnification.

Direct immunofluorescence test (DIF)

The MerilFluor® Criptosporidium/Giardia, Direct Immunofluorescent Detection Procedure, Meridian, Bioscience, Inc. was used. Mixed concentrated sediment (10 μL) was smeared, with the transfer loop, in the well of the slide. 1 drop of detection reagent and 1 drop of counter stain were added, mixed gently, and incubated in a moist chamber. The slide well was rinsed with kit wash buffer. 1 drop of mounting medium was placed to close the cover slip. Wells were examined by fluorescence microscopy with FITC excitation/emission filters.

Therapy

Monil®5% (Pliva d.d., Croatia) containing 50 mg albendazole in 1 ml was used for treatment. The pigs were treated with 20 mg/kg albendazole, *per os*, once daily, for 3 days.

After the therapy faecal samples were sent for parasitological examination (SAF, DIF), to ascertain whether the therapy was effective.

Results

After 3 days of therapy the diarrhoea stopped and the pigs were clinically healthy. They remained without diarrhoea for the next four months till the end of wintering.

No pathogenic bacteria were isolated at the first bacteriological examination on nutrient agar supplemented with 5% of sheep blood and Drigalski agar, in enriched in buffered peptone water and Rappaport Vassiliadis broth, and then subcultured onto solid selective media XLD agar and Rambach agar.

The second examination confirmed the presence of *Campylobacter* coli.

Table 1: Results of the first SAF and DIF examination

	SAF	DIF	
	Giardia sp.	*Cryptosporidium sp.*	*Giardia sp.*
Sow 1	pos.	neg.	pos.
Piglet 1	neg.	neg.	neg.
Piglet 2	neg.	neg.	pos.
Piglet 3	pos.	neg.	pos.
Piglet 4	neg.	neg.	neg.
Sow 2	neg.	neg.	neg.

Giardia sp. was detected by microscopy examination (SAF, DIF) at first during diarrhoea.

Seven days, ten days and eleven days after the treatment all tested samples were negative by SAF and DIF examinations.

Discussion

Giardia spp. and *Cryptosporidium* spp. are commonly identified intestinal pathogens in humans and animals, causing asymptomatic to severe intestinal infections, depending on various factors (8).

Diagnosis of intestinal parasitic disease is confirmed by recovery and identification of protozoan cysts in the parasitological laboratory. The sodium-acetate acetic acid-formalin (SAF) fixative was used as a multipurpose fixative-preservative, permitting the recovery and identification of intestinal parasites for all diagnostic steps (28).

The most widely used assays for detecting *Giardia* and *Cryptosporidium* are the direct immunofluorescence (DIF) assays (28). The sensitivity of the most commonly used commercial DIF test, the MerilFluor® Criptosporidium/Giardia, has been reported to be 95 to 100%, with a specificity of 99.8 to 100%, for both *Giardia* and *Cryptosporidium* (28, 29, 30, 31, 32). This test has a sensitivity greater than the traditional examination of permanent smears for *Giardia* (33) and equal to or greater than that of the traditional examination of permanent smears prepared from concentrated stool specimens for *Cryptosporidium* (30).

Giardiasis is currently treated with metronidazole, tinidazole and quinacrine (34). The adverse effects and treatment failures of some of the currently recommended drugs (particularly 5-nitroimidazoles) for giardia infection have raised the need

for alternative anti-giardia agents. A recent study suggested that two benzimidazole drugs, albendazole and mebendazole, are clinically effective against human giardiosis (35). *In vitro*, albendazole inhibits the growth of trophozoites of *Giardia* and their adhesion to cultured intestinal epithelial cells and disturbs the activity of microtubules and microribbons in the trophozoite's adhesive disk (36). Albendazole was successfully used for treating clinical giardiosis in dogs at a dosage of 25mg/kg *per os*, twice daily, for two days. It was found to be highly effective, 50x more so than metranidazole (37). In productive animal species, albendazol is highly effective for eliminating Giardia in house and range calves (35). In pigs, they treated giardiosis with 30 mg/kg of metronidazole, *per os*, once per day, for 3-5 days. All the contact piglets in the same pen received the same treatment. As a result, the clinical incidence decreased rapidly to 0, 8-1, 3% (38). Under the council Regulation (EEC) 2377/90 (Appendix IV) of 26 June 1990, the use of metronidazole as a veterinary medicine has been prohibited (39) and, for this reason, Albendazole was used for treating our pigs. Treatment with 20 mg/kg albendazole, *per os*, once daily, for 3 days stopped the diarrhoea in all treated pigs, which remained without diarrhoea for the next four months (all the period of wintering). Three weeks after the therapy, *Giardia* was absent from the faeces (negative SAF and DIF). This is the first use of albendazole to be reported for therapy of clinical giardiosis.

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UČINKOVITOST ALBENDAZOLA PRI ZDRAVLJENJU GIARDIOZE PRI PRAŠIČIH

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Povzetek: Giardioza je zajedavska bolezen, ki se kaže v obliki kronične driske in je razširjena tako pri ljudeh kot pri domačih in divjih živalih po vsem svetu. V času prezimovanja prašičev krškopoljske pasme v hlevih Inštituta za zdravstveno varstvo prašičev smo pri dveh plemenskih svinjah in štirih pitancih opazili drisko. Za mikrobiološke preiskave smo uporabili rektalne brise. Vzorci blata so bili pregledana tudi na prisotnost zajedavcev z metodo koncentracije protozojev (SAF) in DIF (direct immuno fluorescent test). Rezultati prvih bakterioloških preiskav niso potrdili prisotnosti nobenih patogenih bakterij, ob ponovni bakteriološki preiskavi pa je bila ugotovljena prisotnost *Campylobacter coli*. Zajedavca *Giardia* sp. smo potrdili z metodo SAF in DIF ob prvi preiskavi, ko je bila prisotna tudi driska. Prašiče smo zdravili z zdravilom Monil®5% (Pliva d.d., Hrvaška) pri čemer je znašal odmerek albendazola 20 mg/kg. Zdravilo je bilo aplicirano per os, enkrat dnevno tri dni zapored. Po tridnevnem zdravljenju driska ni bila več klinično zaznavna in pri zdravljenih prašičih nismo zaznali nikakršnih kliničnih odstopanj. Pri zdrvaljenih prašičih nismo opazili driske še nadaljnje štiri mesece. Sedmi, deseti in enajsti dan po zdravljenju so bili vsi preiskani vzorci blata z metodo SAF in DIF negativni.

Ključne besede: prašič; Giardia; zdravljenje; albendazole

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Case Report

A CASE-CONTROLLED STUDY OF FELV INFECTED CATS IN TEHRAN, IRAN, CONFIRMED BY IMMUNOCHROMATOGRAPHY AND RT-PCR AND CORRELATION WITH CLINICAL AND HEMATOLOGICAL FINDINGS

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Summary: Immunochromatography (ICGA), reverse transcriptase polymerase chain reaction (RT-PCR) and hematological assays were performed on blood samples of 90 cats (45 healthy or control ones and 45 sick or case ones) in Tehran, Iran, as a case-control study from June 2009 through February 2010. Prevalence of FeLV in this population was 1.1% and 2.2% as determined by immunochromatography and RT-PCR assays, respectively. Factors that were significantly associated with positive results in RT-PCR method were pale mucous membrane (P=0.026) and rhinitis (P= 0.002), which were more prevalent in FeLV-positive cats. The size of population of the household was found to be a predictor for FeLV infection, and the relative risk of FeLV infection in cats kept in multicat households is 6.6 times higher in comparison with single housed cats. The most common clinical findings in control group were gingivitis and/or stomatitis (37.8%), skin lesions (8.9%), lymphadenopathy and pale mucous membrane (6.7%), and the most frequent hematological findings were decreased PCV (24.4%), lymphopenia and decreased hemoglobin level (20%), leukocytosis and neutrophilia (13.3%). In the case group, the most common clinical findings were gingivitis and/or stomatitis (77.8%), pale mucous membrane (53.3%) and skin lesions (37.8%), and the most frequent hematological findings were lymphopenia (37.8%), anemia (26.7%), decreased Hb (24.4%) and leukopenia (15.6%).

Key words: cat; feline leukemia virus (FeLV); prevalence; immunochromatography; reverse transcriptase polymerase chain Reaction (RT-PCR)

Introduction

Feline leukemia virus (FeLV) is an oncogenic, myelosuppressive and immunosuppressive γ-retrovirus that occurs throughout the world and represents one of the most important pathogens of domestic cats. FeLV is generally transmitted horizontally in felines by close contact through saliva, blood and other body fluids (1, 2, 3). Risk factors for FeLV infection include gender (more common in males), age, illness and access to outdoor environment, whereas indoor lifestyle and sterilization are associated with reduced infection rates. FeLV infection is the infection of "social cats" because it is mostly spread through social contacts (3, 4). The role of the cat flea

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(*Ctenocephalides felis*) has also been suggested as a possible factor in transmission (5). Reported prevalence of this virus differs considerably depending on the geographical region, the cat population evaluated and especially on the method used in different studies due to differences in sensitivity and specificity of these diagnostic methods. The infection rate of free roaming cats is similar throughout the world, ranging from 1- 8% in healthy cats and up to 21% in sick cats (3). The most recent studies report a prevalence of 2.3-3.3% in North America, 0-14.2% in Asia and 3.5- 40.5% in Europe (6-30). Two previous serologic studies carried out in Iran have shown infection rates between 4.8% to 14.2% in two different regions and different populations of household and stray cats (6, 13). Routine diagnosis screening for FeLV relies on detection of the core viral antigen p27 by ICGA or ELISA, which is produced abundantly in majority of infected cats. In-clinic test kits detect soluble circulating antigen in peripheral blood. Molecular diagnostic methods like polymerase chain reaction (PCR) are becoming more popular due to their advantages over serological methods. The PCR technique is extremely sensitive and the method allows identification of the virus independently of the presence of viremia (31).

According to Iranian society for prevention of cruelty against animals (IRAN, SPCA), more than 90% of cats (*Felis catus*) in Iran are stray and most of owned cats are kept outdoors (6). The aims of this study were to determine the prevalence of FeLV infection by serological and molecular methods among client-owned cats referred to Small Animal Polyclinic, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran (45 healthy or control and 45 sick or case cats). This was the first molecular assay on FeLV in Iran. Seropositivity and PCR positive results were also correlated with clinical and hematological findings such as health status, gender, age and lifestyle.

Materials and methods

Clinical examination

The study group comprised of 90 cats presented to Small Animal Polyclinic, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran from June 2009 through February 2010. These client-owned cats were randomly selected with no limitation for age, gender and lifestyle. These cats were divided into two different groups according to their clinical signs (45 healthy and 45 sick cats). Regarding the most common clinical signs reported in FeLV infected cats, the diagnostic criteria was delineated. These clinical signs included weight loss, fever, dehydration, rhinitis, diarrhea, oral inflammation (gingivitis and/or stomatitis), lymphadenopathy and cutaneous lesions and abscesses (3). Each cat that had two or more of these clinical signs simultaneously was considered as a case cat and those that were presented for vaccination or a routine checkup were considered as control cats. Informed consent was obtained from each cat owner prior to the study. A detailed questionnaire was completed for each animal. Investigated parameters included putative risk factors such as age, gender, breed, health status, sexual intactness and housing conditions (only indoors or outdoors; single or multicat household; to be in contact with other cats or not).

Laboratory examination

Blood samples (2-3 ml) were drawn from jugular or cephalic vein of adult cats (in kittens, only 1 ml of blood was drawn). The collected blood was divided and poured into plain tubes and anticoagulant containing tubes (ethylenediamine tetraacetic acid). Serum samples were separated after centrifugation (for 10 minutes at the speed of 3000 rpm) for serological testing. The separated serum was kept at -20 C° before performing rapid immunochromatography assay. Complete blood counts were performed by automatic cell counter (Nihon Kohden, Tokyo, Japan) for all cats, and parameters such as hematocrit, hemoglobin and platelet counts were rechecked manually according to the guidelines of International Committee for Standardization of Hematology (ICSH). The presence of hematological disorders such as anemia (Hematocrit < 20), leukopenia or leukocytosis (less than 5500 to more than 19,500 leukocyte/µl of blood) and changes in differential leukocyte counts was recorded.

Immunochromatography assay (ICGA.

ICGA was carried out with a commercial kit (Speed Duo® FeLV/FIV, BVT Company, La Seyne sur Mer, France) for detecting p27 antigen of FeLV according to the manufacturer's instructions. Sam-

ples with positive results were retested after a minimum of 30 days according to the guidelines of the American Association of Feline Practitioners' for feline retrovirus management and only considered truly positive if they tested positive for the second time (31). The sensitivity and specificity of the kit in comparison to viral isolation was recorded as 89.1% and 97.7%, respectively.

Reverse transcription polymerase chain reaction

Reverse Transcriptase PCR assay was performed with a commercial kit (VeTeK™ FeLV Detection Kit, iNtRON Biotechnology Inc, Gyeonggi-Do, Korea) for direct detection of feline leukemia virus on the basis of a genetic database.

A commercial kit was used for RNA extraction (VeTeKTM Viral Gene-spin™ Viral DNA/RNA Extraction kit, iNtRON Biotechnology) from 150 µl whole blood according to manufacturer's instructions. Extracted RNA was collected in sterile microcentrifuge tube and stored at -40 C°.

Reverse Transcriptase PCR reaction was carried out according to instructions of the manufacturer. PCR assay were done by using PCR machine (Corbett Research Company, Mortlake, Australia). The amplified products were analyzed in 1.5% agarose gel electrophoresis, using 100bp DNA ladder (Fermentas, Vilnius, Lithuania) as a reference marker. Control RNA of the kit was used as positive control and distilled water as negative control. Primers used in this kit were able to amplify a 239bp segment of FeLV genome. Therefore, samples that had a 239bp segment of FeLV genome were considered as positive (Figure 1).

Statistical analysis

Cases positive with immunochromatography and positive with RT-PCR were set as outcome variables, while the independent variables were sex, age, health status (including most prevalent clinical signs stated above), population of the household, sexual status and abnormal hematological findings (e.g. anemia, leukopenia, leukocytosis, etc.). Prevalence was calculated as the percentage of cats with positive ICGA and PCR results. Asymptotic χ^2, Mann-Whitney U and independent sample t-test were used to test the bivariate associations between each of the putative risk factors and infection. Risk factors found to be significantly associated with risk of infection in bivariate analyses were included in logistic regression analyses. For these analyses, categorical variables were recorded as indicator variables. Regression models were built by analyzing the main effects of covariates using a forward selection procedure, with a P-value for the likelihood ratio test of <0.05 used for selection. All statistical analyses were performed with standard software (SPSS 15.0 for Windows, SPSS, Chicago, Illinois, USA). Values of $P<0.05$ were considered to indicate a statistically significant difference.

Results

In the present study, only 1 cat out of 90 cats (1.1%) tested positive for the presence of FeLV antigen, and 2 cats from this population (2.2%) were positive in RT-PCR assay, and no cat was positive by both methods simultaneously. Whereas all positive cats belonged to the case group, the overall prevalence of FeLV in control group with ICGA and PCR was 0%. Overall prevalence of FeLV infection in case group was 2.2% and 4.4% by ICGA and PCR methods, respectively. Source, health status and abnormal clinical and hematological findings of FeLV positive cats were summarized (Table 1). According to results of this study, all positive cats belonged to DSH breed and were younger than 3 years old. Two cats out of 3 positive cats (66.7%) had free access to outdoor. The same percentages of these positive cats were sexually intact and kept in multicat households. Most common abnormal clinical findings in FeLV positive cats were pale mucous membranes (100%) and gingivitis/Stomatitis (66.7%). No cat was positive in two tests simultaneously. Regression analysis confirmed these factors as significant risk factors for FeLV infection. The full logistic regression model containing selected predictors, without interactions, was statistically significant (LR $\chi^2=150.87$, $P<0.001$, likelihood=17.50).

Factors that significantly associated with positive cats in PCR assay by Asymptotic χ^2 test, were pale mucous membrane ($P=0.026$) and rhinitis ($P=0.002$), which were more prevalent in FeLV positive cats (Table 2). The comparison of quantitative variables between positive and negative cats in PCR assay by Mann-Whitney-U test showed that only the difference of eosinophil count was statistically significant between two groups and it was higher in infected cats ($P=0.043$).

Table 1: Source, signalment, health status, and abnormal clinical and hematological findings of FeLV positive cats (3 positive results out of 90 samples).

Age	Gender	Breed	Lifestyle	Type of household	Sexual status	Abnormal clinical findings	Abnormal hematological findings	FeLV ICGA	FeLV PCR
1Y	F	DSH	Indoor/ Outdoor	Multicat	Intact	Fever, Diarrhea, Pale mucous membranes	Leukocytosis, Neutrophilia	+	-
10 month	M	DSH	Indoor	Single	Neutered	Gingivitis/Stomatitis, Lymphadenopathy, Pale mucous membranes	Anemia, Thrombocytopenia	-	+
3Y	F	DSH	Indoor/ Outdoor	Multicat	Intact	Gingivitis/stomatitis, Rhinitis, Pale mucous membranes	Thrombocytopenia	-	+

Y= year, M= male, F= female, DSH= domestic shorthair

Table 2: A comparison of the qualitative variables between positive and negative cats in PCR by asymptotic χ^2 test

Variable	χ^2	df	P Calculated
Gender	0.006	1	0.936
Population of household	0.06	1	0.807
Sexual status	0.486	1	0.486
Diarrhea	0.122	1	0.727
Weight loss	0.289	1	0.591
Gingivitis/ Stomatitis	1.524	1	0.217
Lympha_ denopathy	2.055	1	0.152
Pale mucous membrane	4.958	1	0.026 *
Cutaneous abscesses and lesion	0.427	1	0.094
Rhinitis	9.87	1	0.002 *
ICGA positive result	0.023	1	0.879

*Statistically significant difference

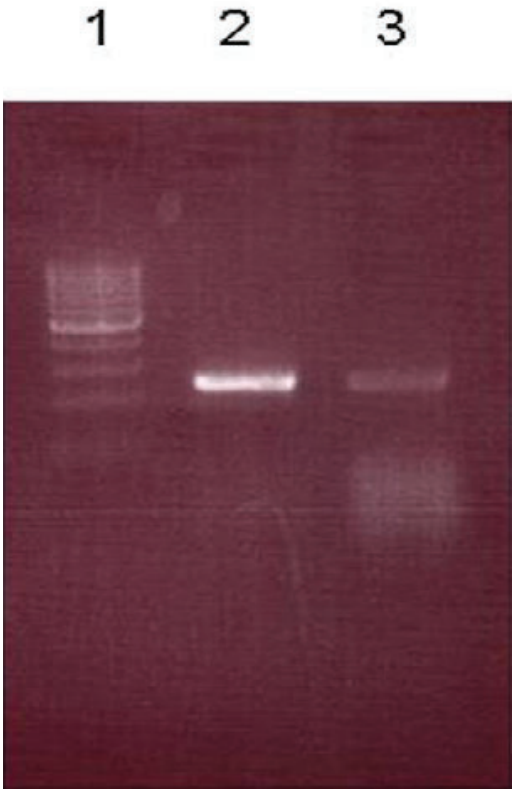

Figure 1: The positive band (column 3) beside positive control of the RT-PCR kit (column 2) and 100 bp ladder (column 1) in gel-electrophoresis

Discussion

The current study revealed an overall prevalence of 1.1% and 2.2% for infection with FeLV in Tehran, Iran, by serologic and molecular methods, respectively. Previous studies performed in Iran revealed infection rates between 1.6% to 14.2% in two different regions and different populations of household and stray cats. In the first study in Iran (Tehran), among 103 healthy domestic and stray cats, 4.8% showed positive serologic results for FeLV through ELISA method but there was no molecular analysis performed in this study (13). In another study conducted in southern Iran (Kerman) on household and stray cats, serum infection was reported to be about 14.2% (6). Our estimated prevalence is in accord-

ance with the study performed in Tehran in 2008; however, it is obviously different from the results of the study conducted in Kerman, though the same commercial ICGA kit was used for serologic evaluation in the latter. It seems that the prevalence of retroviral infection represents obvious regional patterns in some countries (8). This pattern may also be present in different parts of Iran. On the other hand, the population that was studied in Kerman comprised 70 urban stray cats. Our study was a case-controlled study which was performed on 90 client-owned cats, 42.2% of which were kept individually and 35.5% of which had no access to outdoor. This low prevalence rate may have different reasons. Firstly, it could be due to the low prevalence of FeLV in Tehran. Another probable reason may be "latent" cats, that is, cats which are permanently infected with FeLV but have no detectable antigen in their peripheral blood. Evaluating bone marrow for existence of FeLV genome is required to detect such cats, but this was not done in the present study. Prevalence of FeLV infection in cat populations differs throughout the world. The reported seroprevalence for FeLV in healthy and sick client-owned and free-roaming cats that are in accordance with our estimated prevalence were 0% in Vietnam (8, 18), 2% in Australia (15, 16), 1.3% in Taiwan (17), 2.9% in Japan (11) 3% in Switzerland (19) 1% in Finland (20) and 2.3% in USA (21). The 35.7% prevalence rate was reported in the study performed by nested PCR on 179 blood samples in Spain (22). In another study, conducted in England, 21.72% of the blood samples were positive by quantitative PCR method (30) Tozon et al. reported 17 positive cats out of 42 cats by PCR method (28).

Interestingly, no cat was positive in both tests. Although the RT-PCR test is very sensitive, retroviruses, including FeLV, are usually genetically variable, and therefore it is possible that the primer used by our RT-PCR kit were not able to detect all strains of this virus. Another reason for observed discrepancy might be relatively low sensitivity of immunochromatography test that may lead to some false negative results.

In regard to low seroprevalence of FeLV by ICGA (only 1 positive sample), it was inapplicable for statistically comparing the characteristics and abnormal clinical and hematological findings of FeLV positive and FeLV negative cats in this study, and there was no statistically significant difference between positive and negative cats in regard to the serological method. As a descriptive statistic, all three positive cats in our study were younger than 3 years old (from 10 months to 3 years old). It was believed that the susceptibility of the cats to FeLV was age-dependent, and that younger cats were more susceptible to FeLV infection (3, 4, 14). Pale mucous membranes (100%) and gingivitis or stomatitis (66.7%) were the most common clinical findings in FeLV positive cats in our survey, what is consistent with other reports, but these differences were not statistically significant (24).

Factors that were significantly associated with positive RT-PCR results were pale mucous membrane (P=0.026) and rhinitis (P=0.002), which were more prevalent in FeLV positive cats. In a similar comparison based on Mann-Whitney-U Test made with the cats that were positive in RT-PCR test, it was shown that the presence of eosinophilia can increase the probability of positive results in the RT-PCR test. This factor has not been mentioned in previous studies, and since the number of PCR positive cases was very low in our study, this might be due to a chance. By using the full logistic regression model, the population of the household was found to be a predictor of FeLV infection, and the relative risk of FeLV infection in cats kept in multicat households was 6.6 times higher in comparison with single cats. Keeping cats in permanent contact with other cats increases the chance for contact with other possibly infected cats; as a result, it increases the overall prevalence of FeLV infection (1, 4, 23). The most prevalent hematological abnormalities in FeLV-infected cats were thrombocytopenia (66.7%), anemia and leukocytosis (33.3%), which were reported by other authors (23), but in some other studies, leukopenia and lymphopenia were reported to be more prevalent (9). According to the results of this study, all positive cats belonged to DSH cross-breed. The same results were described in some other studies (24, 26). This may be related to the higher population of cross-breed DSH cats in many countries such as Iran. Two cats out of three positive cats (66.7%) had free access to open space, but the difference was not significant, even though there was a statistically significant association between the lifestyle and risk of FeLV infection in some previous studies (8, 20, 27, 28, 29).

Finally, according to the low prevalence of FeLV in both case and control cats, it seems that a proportion of this population under study, especially cats with abnormal clinical signs, may be infected with FeLV, but they are in the latent stage of the disease. Evaluating bone marrow samples for existence of

FeLV would be required to confirm this type of infection. Another possible explanation is that these cats may have been infected with other pathogens that are able to display similar clinical signs as FeLV (e.g. Feline immunodeficiency virus, Feline panleukopenia virus). Interestingly, since we used combo immunochromatography kits which are also able to detect the presence of antibodies against FI, we found a relatively high prevalence of FIV in these samples. Sixteen out 90 blood samples (17.8%) were positive for anti FIV antibodies while no cat was positive for FeLV and FIV, simultaneously, and this could perhaps explain some clinical signs. In conclusion, results of ICGA and PCR methods suggest that the overall prevalence of FeLV is very low in Tehran, in contrast to its relatively high prevalence in southern Iran (Kerman). According to the full logistic regression model, the whole model was statistically significant, and if all these putative factors can be mentioned simultaneously, it is possible to predict correctly the FeLV infection status in 94.4% of the cats.

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PREISKAVA OKUŽENOSTI MAČK V TEHERANU Z VIRUSOM FELV Z IMUNOKROMATOGRAFIJO IN RT-PCR TER KLINIČNI IN HEMATOLOŠKI REZULTATI

B. Bardshiri, S.M. Rafie, M.R.S.A. Shapouri, Z. Khaki, B. Akhtardanesh, A. Komeilian

Povzetek: V raziskavi v Teheranu (Iran) so bili med junijem 2009 in februarjem 2010 odvzeti krvni vzorci 90 mačkam (45 zdravim oz. kontrolnim in 45 bolnim) in pregledani z imunokromatografijo (ICGA), obratno transkripcijo in verižno reakcijo s polimerazo (RT-PCR) ter hematološkimi preiskavami. S pomočjo imunokromatografije in RT-PCR so ugotovili, da je prevalenca okuženosti s FeLV v preiskovani populaciji 1,1- in 2,2-odstotna. Dejavniki, ki so bili statistično značilno povezani s pozitivnimi rezultati, dobljenimi z metodo RT-PCR, so bili blede mukozne membrane (p = 0,026) in vnetje nosne sluznice (p = 0,002), ki je bilo bolj pogosto pri mačkah, pozitivnih na FeLV. Okuženost z FeLV se je pojavljala pogosteje v gospodinjstvih z večjim številom mačk. Relativno tveganje za okužbo z virusom FeLV pri mačkah, ki so živele v gospodinjstvih z več mačkami, je bilo 6,6-krat višje, kot pri mačkah, ki so bile v gospodinjstvu same.
V kontrolni skupini so bili najpogosteje ugotovljeni naslednji klinični znaki: vnetje dlesni in/ali želodca (37,8 %), poškodbe kože (8,9 %), limfadenopatija in bledost mukoznih membran (6,7 %). Najpogostejše hematološke ugotovitve so bile: zmanjšan PCV (24,4 %), limfopenija, zmanjšana raven hemoglobina (20 %) ter levkocitoza in nevtrofilija (13,3 %). V skupini okuženih živali so bili najpogostejši klinični znaki vnetje dlesni in/ali želodca (77,8 %), bledost mukoznih membran (53,3 %) in poškodbe kože (37,8 %), najpogostejše hematološke ugotovitve pa limfopenija (37,8 %), anemija (26,7 %), znižana raven hemoglobina (24,4 %) in levkopenija (15,6 %).

Ključne besede: mačka; virus mačje levkoze (FeLV); prevalenca; imunokromatografija; obratna transkripcija in verižna reakcija s polimerazo (RT-PCR)

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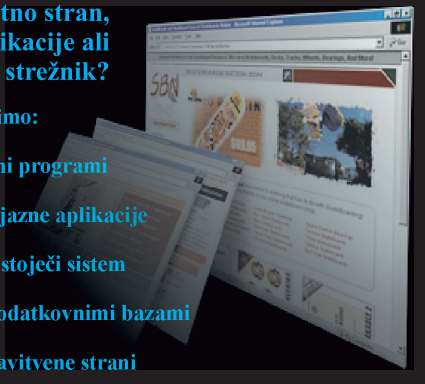

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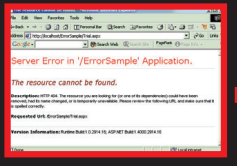

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