

INCIDENCE AND PREVALENCE OF TOXOPLASMOSIS AMONG SHEEP AND GOATS IN SOUTHERN AND WESTERN BOHEMIA

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Abstract

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Incidence and prevalence of toxoplasmosis in several flocks of sheep and goats in southern and western parts of Bohemia were studied, together with some factors influencing the infection.

From 1982 to 1989, a total of 899 slaughter sheep was examined in the Strakonice abattoir, 780 of them from small privately-owned flocks and 119 from larger flocks of agricultural cooperatives. Antibodies to *T. gondii* were detected by Sabin-Feldmann dye test (DT) in 54.6% (56.5% and 42.1%), by complement fixation reaction (CFT) in 40.1% (43.8% and 28.1%) and by microprecipitation in agar gel (MPA) in 3.6% (3.8% and 1.7%). *T. gondii* was detected in 4.8% (5.4% and 1.7%). Both seroprevalence and *T. gondii* incidence were higher among sheep from small, privately-owned flocks than among sheep from cooperative farms. Levels of antibodies detected by DT and CFT remained very much the same throughout the study period. Prevalence of DT and CFT antibodies was significantly ($P < 0.05$, $P < 0.005$ resp.) higher in spring and autumn than in summer and winter.

In 1986-1990, 341 blood serum samples from two small private flocks in the Strakonice district were examined. The DT was positive in 67.1%, CFT in 22.5% and MPA in 0.7%. The difference between the two flocks in incidence of antibodies detected by DT was significant. The incidence of antibodies remained on practically the same level over the entire period. Higher antibody levels were found in sheep examined in spring and summer, but they were not statistically different from levels ascertained in autumn and winter.

Repeated biological semen examination of eleven rams, seven of which were positively DT-tested for antibodies, failed to prove the presence of *T. gondii*.

In 1981-1990, a total of 54 blood serum samples from five small goat flocks were examined. Antibodies were detected by DT and CFT in 61.1% and 21.0% respectively. In all the five flocks, antibodies were detected by DT. Their levels peaked in summer and autumn.

A serological examination of 125 sheep from a military training area in southern Bohemia revealed antibodies in 45.6% and 12.8% by DT and CFT, respectively. DT and CFT performed on 196 sheep from a military training area in western Bohemia revealed seropositivity in 73.9% and 21.9% respectively.

The results showed that the prevalence of toxoplasmosis among sheep and goats in areas investigated is considerable. Its prevalence among sheep in small, privately-owned flocks is higher than in larger flocks of agricultural enterprises. Antibodies to toxoplasmosis tend to stay in sheep for a long time and they are found much more frequently than their causative agent can be detected in tissues. In none of the sheep or goat flocks examined, clinical expressions of toxoplasmosis were observed.

Toxoplasma gondii, sheep, goats, Sabin-Feldmann dye test (DT), complement fixation test (CFT), microprecipitation in agar gel (MPA), monitoring, husbandry.

Toxoplasmosis among sheep and goats has been reported from all over the world (Blewett and Watson, 1984; Dubey and Beattie, 1988). In our circumstances, the main source of *T. gondii* among sheep and goats is the domestic cat. Its faeces containing toxoplasmosis oocysts contaminate either the facilities where animals are kept, their feed (Plant et al. 1974, McSparran et al. 1985, Nurse and Lenghaus, 1986) or bedding (Faul et al. 1986). An intrauterine transmission from one sheep to another is also the frequent type of transfer. The result is either an abortion or the birth of congenitally infected young (Bartko 1979, Arnaudov et al. 1976, Nicolas et al. 1978, Blewett et al. 1982, Calamel 1982, Dubey and Welcomme, 1988, inter alia). Similarly, an intrauterine transfer of *T. gondii* among goats has been described (Calamel and Giauffret, 1975, Munday and Mason, 1979, Plant et al. 1980, Dubey et al. 1980, 1985, Dubey 1981, 1982, and others). Spence et al. (1978) and Teale et al. (1982) reported excretion of *T. gondii* in the semen of experimentally infected rams. Blewett et al. (1982), however, failed to demonstrate a transmission of *T. gondii* to healthy sheep tupped by rams with chronic toxoplasmosis. Dubey and Sharma (1980) demonstrated long-term excretion of *T. gondii* in semen after experimental infection with toxoplasma oocysts. Agent transmission from experimentally infected sheep to healthy ones during their contact was not demonstrated (Bicknell 1972, Michael et al. 1972), although Beverley et al. (1975) pointed out that oocysts of *T. gondii* may pass through the digestive tract without opening up, which

would create an opportunity for the spreading of oocysts in the faeces of infected sheep. Similarly, Owen and Chesum (1975) stressed the importance of amniotic fluid and amnions in toxoplasma abortions as a possible source of *T. gondii*. Schurian (1969) and Ruppner et al. (1978) failed to demonstrate toxoplasmosis agent excretion in milk of latently infected sheep and goats respectively. Riemann et al. (1975), on the other hand, demonstrated *T. gondii* in goat milk. In goats experimentally infected with a large number of oocysts, Dubey (1980) reported excretion of *T. gondii* in milk only exceptionally and he believes that the risk of toxoplasmosis incidence in milk of naturally infected goats is minimal.

In monitoring toxoplasmosis incidence and prevalence among sheep and goats, attention was also paid to other factors influencing its epizootiology. Calamel (1982) and Waldeland (1976) concluded that toxoplasmosis affected flocks of sheep and goats with no geographical influence. Later, however, Waldeland (1977) reported that toxoplasmosis was more frequent among animals from lowland pastures, and more frequently in winter than in summer, than among animals on mountain pastures in uninhabited countryside. Arnaudov et al. (1976), on the other hand, found higher seropositivity among sheep in mountainous areas than in lowlands. Dubey and Welcom (1988) found a higher percentage of serologic reactions to toxoplasmosis in older sheep. Higher seropositivity was also found among older goats (Machado and Lima 1987, Dubey and Adams 1990). Husbandry-related differences in toxoplasmosis incidence have also been reported. Machado and Lima (1987) found the highest seropositivity among goats raised for meat and milk, lower among dairy goats raised on private farms, and the lowest among goats raised intensively for meat. Chiari et al. (1987) found a significantly higher percentage of antibodies among goats raised in towns and neighbouring areas than in the country. Machado (1985) demonstrated higher seropositivity among goats on private farms than on dairy farms or extensive breeding farms.

The aim of the present study was to investigate the incidence and prevalence of toxoplasmosis among sheep and goats in southern and western Bohemia as a part of a comprehensive study of animal toxoplasmosis, and to identify influences that affect the occurrence and spreading of the infection.

Materials and Methods

A. Between 1982 and 1990, the following examinations were made in a south Bohemian district of Strakonice:

1. Between 1982 and 1989, samples of blood, brain and diaphragm muscle were collected in one month intervals from slaughtered sheep. The samples were collected from 10 to 15 sheep as they were slaughtered on that day. Most of the sheep were from small, privately-owned flocks, the rest from larger flocks raised by local agricultural enterprises.
2. Between 1986 and 1990, blood samples were taken regularly in three-month intervals from two small flocks of sheep.
3. In 1984, semen of 11 rams from a large flock of a local agricultural enterprise was taken several times in 17 day-intervals.
4. In 1981-84, blood samples of goats from two small flocks were taken occasionally, and in 1986-1990, blood samples of goats from three small flocks were taken regularly, as in the case of sheep.

B. In 1987 and 1988, blood samples of sheep raised in large flocks in two military training areas in southern and western Bohemia were taken.

Serological examinations to demonstrate the presence of antibodies against *T. gondii*, DT (titre \geq 4), CFT (titre \geq 10), MPA and an isolation experiment of the causative agent from tissues, were made in the same way as in cattle (Hejlicek and Literak, 1992).

The ram semen was diluted with sterile physiological saline and antibiotics in 1:2 ratio, and applied intraperitoneally in 1 cm³ doses to white mice. Further examinations were the same as in the tissue assay.

Statistical analysis was done using the χ^2 test.

Results

A. Strakonice district examinations

1. A total of 899 slaughter sheep were examined, 780 of which were from small, privately-owned flocks and 119 from larger flocks of agricultural enterprises (Tab. 1). DT was used to examine

Table 1
Incidence of antibodies to toxoplasmosis and demonstration of *T. gondii* in tissues of slaughter sheep in Strakonice region from 1982 to 1989

(DT = Sabin-Feldmann dye test, CFT = complement fixation test, MPA = microprecipitation in agar gel)

Origin of animals	Percent of positive sheep			
	SD (titre \geq 4)	CFT (titre \geq 10)	MPA	<i>T. gondii</i> isolation
Small, privately-owned flocks (n = 780)	56.5 ^a (n = 767)	43.0 ^b (n = 388)	3.8 ^c (n = 627)	5.4 ^d (n = 670)
Large flocks from ag enterprises (n=119)	42.1 ^a (n = 119)	28.1 ^b (n = 96)	1.7 ^c (n = 59)	1.7 ^d (n = 116)
Total (n = 899)	54.6 (n = 886)	40.1 (n = 484)	3.6 (n = 686)	4.8 (n = 786)

a) significant difference ($\chi^2 = 8.2$, df = 1, P<0.005)

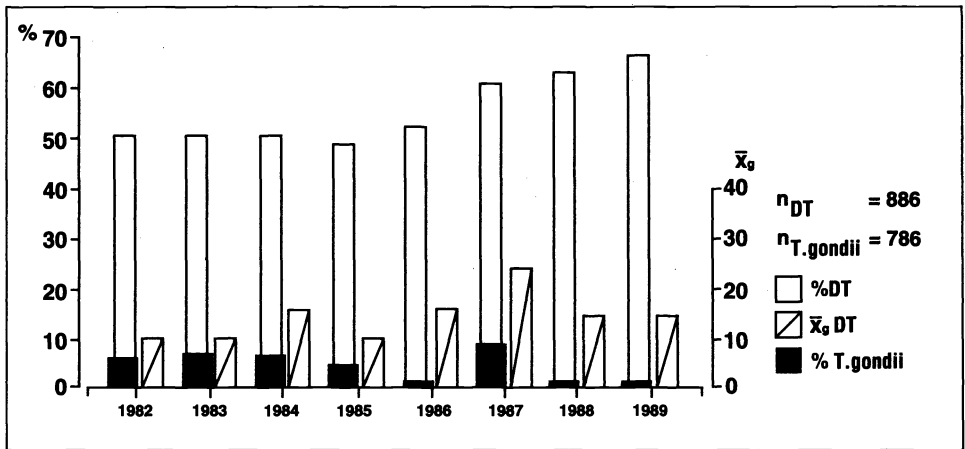
b) significant difference ($\chi^2 = 6.6$, df = 1, P<0.05)

c, d) non-significant (χ^2 test, Fisher test, P>0,05)

886 sheep (767 and 119), of which 54.6% (56.5% and 42.1) tested positive; CFT was used in 484 sheep (388 and 96), of which 40.1% (43.0 and 28.1%) tested positive. MPA was used in 686 slaughter sheep (627 and 59), of which 3.6% (3.8% and 1.7%) tested positive. The presence of *T.gondii* was demonstrated in 4.8% (5.4% and 1.7%) of the 786 (670 and 116) sheep examined.

Incidence of antibodies detected by DT throughout the entire study period (Fig. 1), as well as the geometric mean of titres (\bar{x}_g), remained relatively unchanged. The demonstration of *T.gondii* from tissues peaked in 1987. The incidence of CFT-tested antibodies reached its highest level in 1988, although \bar{x}_g remained the same.

Fig. 1
Prevalence of antibodies to *T. gondii* and incidence of *T. gondii* among sheep slaughtered in the Strakonice abattoir in individual seasons of the year (1982–1989) (CFT complement fixation test)



Prevalence of DT- and CFT-tested antibodies was significantly different in individual seasons of the year ($\chi^2_{DT} = 8.5$ df = 3 $P < 0.05$; $\chi^2_{CFT} = 11.2$ df = 3 $P < 0.005$) (Fig. 2a, 2b). An increase was observed in spring and autumn, and a decrease in winter and summer. No significant changes in tissue assays of *T.gondii* in individual seasons were found.

2. A total of 341 blood samples were taken from sheep in two small, privately-owned flocks (Tab. 2), of which 340 samples were tested by DT (67.1% positive), 329 were tested by CFT (22.5% positive) and 298 serum samples were tested by MPA (0.7% positive). The two flocks differed significantly in the levels of antibodies detected by DT, while no significant differences in the levels of antibodies detected by CFT or MPA were found.

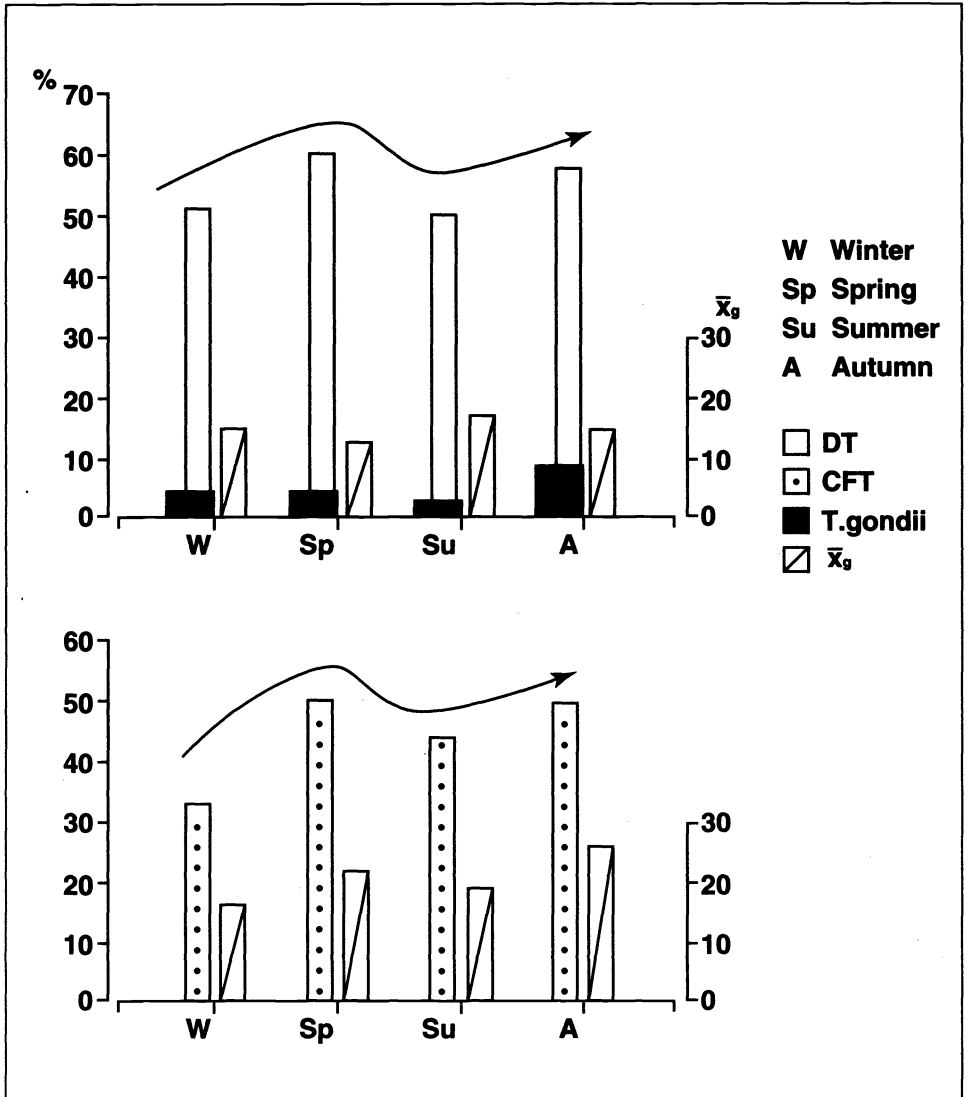
Table 2
Prevalence of antibodies to toxoplasmosis among sheep in two small privately-owned flocks in Strakonice district

Farm	Monitoring Period	Percent of positive sheep		
		DT	CFR	MPA
1 (S. R.)	1986–1990	78.1 ^a (n = 201)	25.2 ^b (n = 198)	1.1 (n = 175)
2 (K. N.)	1986–1990	53.2 ^a (n = 139)	18.3 ^b (n = 131)	0 (n = 123)
Total	1986–1990	67.9 ^{aa} (n = 340)	22.5 ^{bb} (n = 329)	0.7 (n = 298)

a) significant difference ($\chi^2 = 22.3$, 1df, $P < 0.001$)
 b) non-significant difference (χ^2 test, $P < 0.05$)
 aa) non-significant (sheep:goat) (χ^2 test, $P < 0.05$)
 bb) non-significant (sheep:goat) (χ^2 test, $P < 0.05$)

Fig. 2

Prevalence of antibodies to *T. gondii* and incidence of *T. gondii* among sheep slaughtered in the Strakonice abattoir in individual years (DT = Sabin-Feldmann dye test
 \bar{x}_g = geometric mean of titre)



The prevalence rate of antibodies changed little in individual years (between 1986 and 1990), with the highest percentage being recorded in 1988. DT and CFT detected antibodies more frequently in spring and summer, but the difference from other seasons (autumn and winter) was not significant.

3. Blood serum antibodies were found in seven of the eleven DT-tested rams. The titres were from 8 to 64. Biological experiments with repeatedly taken semen, however, failed to demonstrate the presence of *T. gondii*.

4. A total of 54 blood serum samples from goats in five small, privately-owned flocks was examined (Tab. 3) DT and CFT detected antibodies in 61.1% and 21.0% respectively. Antibodies were found in all five flocks where DT were used. The highest percentages of positive results in DT and CFT were recorded in summer and, somewhat lower, in autumn.

Table 3
Prevalence of antibodies to toxoplasmosis among goats in five small, privately-owned flocks in Strakonice district

Farm	Monitoring period	No. of positive (No. of examined goats)		
		DT	CFR	MPA
1 (M. V.)	1981-1984	2/10	nt	nt
2 (R. V.)	1981-1984	4/5	nt	nt
3 (L. T.)	1986-1990	16/22	6/22	0/20
4 (A. K.)	1986-1990	2/2	0/2	0/1
5 (K. N.)	1986-1990	9/15	2/14	0/13
Total	1981-1990	33/54 ^{aa} (61.1 %)	8/38 ^{bb} (21.0 %)	0/34 (0 %)

nt = not serologically tested

aa) no significant (sheep:goat) (χ^2 test, $P>0.05$)

bb) no significant (sheep:goat) (χ^2 test, $P>0.05$)

B. Examinations in two military training areas

In a military training area in south Bohemia, the authors examined 125 sheep, which tested positive by DT and CFT in 45.6% and 12.8% respectively. Of the 196 sheep examined in a west Bohemian training area, 73.9% and 21.9% tested positive by DT and CFT respectively.

Discussion

In 1982-89, a total of 899 sheep slaughtered in the Strakonice abattoir were tested for toxoplasmosis. DT tested positive in 54.6%, CFT in 40.1%, MPA in 3.6% and an isolation experiment in 4.8%. These findings are in a general agreement with results obtained in toxoplasmosis examinations in sheep in Czechoslovakia (DT 80%, CFT 26%, isolation experiment 13%), as summarized for a period of 1948-1970 by Kouba et al. (1974). They are, however, considerably higher than those reported for DT by Surveillance of Antropozoonosis in the Czech Republic for 1979-1990 (22.7%) and those reported for CFT from the Slovak Republic for the 1988 - 1991 period (9.7%) by Kováčová (1993). Higher findings in our group of slaughter sheep could be explained by the fact that this region has a markedly higher incidence of antibodies to toxoplasmosis also in beef cattle (4.1%, Hejlíček and Liteřák, 1992), compared to the 1979-1990 average in the Czech Republic (1.4%) reported by Surveillance of Antropozoonosis. Besides, our group consisted mostly of sheep from small, privately-owned flocks (780 out of 899), where the risk of toxoplasma oocyst infection is higher than in larger flocks that are raised in sheep-pens and on pastures. This is also demonstrated by a comparison of results of examinations in small, privately-owned sheep flocks and large flocks from agricultural cooperatives, where positive results of DT and CFT in the former were significantly higher. Levels of positive results of DT and \bar{x} g remained practically the same over the years, which is a proof of a permanent occurrence of toxoplasma oocysts in the environment excreted by the domestic cat, and of a long prevalence of antibodies to toxoplasma in infected sheep, which was already pointed out by B l e w e t t (1983). This might also explain a difference between the high DT-detected seropositivity (54.6%) and a relatively small number of positive demonstrations of agents in tissues (4.8%). Similar findings in slaughter sheep were also reported by Punke (1973).

A significantly higher prevalence of antibodies detected by DT and CFT was found among sheep slaughtered in spring and autumn. In spring, a newly born feline population, which

is very sensitive, is infected and excretes oocysts in faeces. Higher incidence of antibodies in autumn may be related to the fact that mostly old and culled sheep, which are more frequently infected with *T. gondii*, are slaughtered at that time. This assumption is supported by a more frequent identification of *T. gondii* in tissues of sheep examined in autumn. A higher percentage of serologic responses in older sheep was also reported by D u b e y and W e l c o m e (1988).

The five-year serologic study of sheep in two privately-owned flocks revealed significant differences in DT-positivity. This is apparently related to the local epizootiological situation in source availability. Average seropositivity in sheep from the two flocks was not markedly different from that among slaughter sheep from small privately-owned flocks. Just as in slaughter sheep, no significant differences in prevalence of antibodies to toxoplasma or geometric mean of titres in individual years were observed. Incidence of antibodies in individual years did not vary significantly, with the highest percentage being reached in spring and summer. This was apparently due to the contamination of the environment with oocysts from a newly born population of cats. Lower autumn prevalence of antibodies in comparison with slaughter sheep can be explained by the fact that, rather than monitoring only older or culled sheep, all sheep in the flock were monitored. This pattern of prevalence of antibodies in small flocks, with its peaks in spring and summer, may be considered quite representative, and it clearly identifies the role of cats, particularly younger ones, in influencing the epizootiological situation in sheep toxoplasmosis.

A repeated biological assay of the ejaculum of 11 rams, seven of which tested positive by DT, failed to prove the presence of *T. gondii*. Therefore, we were not able to confirm the report of *T. gondii* excretion in ram semen, as published by S p e n c e et al. (1978) and T e a l e et al. (1982). These authors, however, examined experimentally infected rams. Results of our study seem to be more in agreement with observations of B l e w e t t et al. (1982), who failed to demonstrate a transfer of *T. gondii* to healthy sheep tupped by rams with chronic toxoplasmosis. No increase in the number of abortions was observed in the flock where 11 rams were examined.

A total of 54 blood samples was taken in five small goat flocks. With regard to the incidence of antibodies (DT 61.1%, CFT 21.0%), no significant difference was found from the number of positive samples from small, privately-owned sheep flocks. Although only a small number of samples was examined, antibodies were found most frequently in summer and autumn. Compared to sheep in small flocks where the prevalence of antibodies reached the highest level in spring and summer, this represents a certain shift. It can be explained by the fact that goats in that region are brought to pastures later in spring than sheep, which delays their infection with toxoplasma oocysts.

To provide more background information on the incidence and prevalence of toxoplasmosis among sheep in the Strakonice district, we made a single examination of sheep from two different locations in southern and western Bohemia. They were both military training areas, sparsely populated and with little production activity. High seropositivity in sheep raised in those areas can be explained by the presence of cats in an immediate vicinity of sheep pens, a high sensitivity of sheep to infection and a long survival of antibodies in blood. The areas are large and sparsely inhabited, and a contamination of pastures with oocysts of *T. gondii* is therefore unlikely.

No increase in the number of abortions was observed in either of the flocks.

Výskyt a rozšíření toxoplazmózy u ovcí a koz v jižních a západních Čechách

Byl sledován výskyt a rozšíření toxoplazmózy v chovech ovcí a koz na území jižních a západních Čech, spolu s některými faktory ovlivňujícími nálezovou situací.

В roce 1982–1989 bylo vyšetřeno na jatkách ve Strakonících 899 jatečných ovcí, z toho 780 z malých soukromých chovů a 119 z větších chovů zemědělských závodů. Protilátky proti *T. gondii* byly prokázány SFR (Sabin-Feldmanova reakce) v 54,6 % (56,5 % a 42,1 %), KFR (komplementfixační reakce) ve 40,1 % (43,8 % a 28,1 %), MPA (mikroprecipilace v agarovém gelu) ve 3,6 % (3,8 % a 1,7 %). Přítomnost *T. gondii* byla zjištěna ve 4,8 % (5,4 % a 1,7 %). Séroprevalence i výskyt *T. gondii* byly vyšší u ovcí v malých, soukromých chovech než ve větších chovech zemědělských závodů. V jednotlivých letech zůstávaly nálezy SFR a KFR protilátek celkem rovnoměrné. Výskyt SFR a KFR protilátek byl na jaře a na podzim signifikantně vyšší než v létě a v zimě.

V roce 1986–1990 bylo vyšetřeno ve dvou malých, soukromých chovech na okrese Strakonice 341 vzorků krevního séra ovcí. SFR byla pozitivní v 67,1 %, KFR ve 22,5 %, MPA v 0,7 %. Mezi oběma chovy byl signifikantní rozdíl ve výskytu SFR protilátek. Výskyt protilátek v průběhu jednotlivých let byl vcelku vyrovnaný. Vyšší nález protilátek byl u ovcí vyšetřovaných na jaře a v létě, avšak signifikantně se od nálezů na podzim a v zimě nelišil.

Při opakovaném vyšetření semene 11 beranů, z nichž 7 bylo se SFR protilátkami, se biologickým pokusem nepodařilo prokázat *T. gondii*.

V roce 1981–1990 bylo vyšetřeno v 5 malých chovech 54 vzorků krevního séra koz. Výskyt SFR a KFR protilátek činil 61,1 % a 21,0 %. Ve všech 5 malých chovech byly zjištěny u koz SFR protilátky. Nejvyšší výskyt protilátek byl v létě a pak na podzim.

Sérologickým vyšetřením 125 ovcí ve vojenském výcvikovém prostoru na území jižních Čech byly zjištěny SFR protilátky ve 45,6 %, KFR ve 12,8 %. Ve vojenském výcvikovém prostoru v západních Čechách bylo vyšetřeno 196 ovcí, z toho SFR pozitivita činila 73,9 %, KFR 21,9 %.

Výsledky prokázaly, že toxoplazmóza ovcí a koz je ve sledovaných lokalitách značně rozšířena. U ovcí je vyšší výskyt v malých, soukromých chovech než ve větších chovech zemědělských závodů. Protilátky proti toxoplazmóze mají tendenci u ovcí dlouho přetrvávat a nacházejí se výrazně častěji, než se daří prokazovat původce ve tkáních. V žádném ze sledovaných chovů ovcí a koz nebyly pozorovány klinické projevy toxoplazmózy.

Наличие и распространение токсоплазмоза овец и коз в южной и западной Чехии

Проводили исследования наличия и распространения токсоплазмоза овец и коз на территории южной и западной Чехии совместно с некоторыми факторами, оказывающими влияние на заражение.

В 1982–1989 гг. на скотобойне в Страконицах исследовали 899 убойных овец, из этого 780 овец из небольших частных овцеводческих хозяйств и 119 из крупных овцеводческих заводов. Антитела против *T. gondii* были выявлены SFR 54,6 % (56,5 % и 42,1 %), KFR 40,1 % (43,8 % и 28,1 %), MPA 3,6 % (3,8 % и 1,7 %). Наличие *T. gondii* было установлено в 4,8 % (5,4 % и 1,7 %). Серопревалирование и наличие *T. gondii* были больше у особей небольших, частных овцеводческих хозяйств чем у овец крупных овцеводческих ферм. В отдельные годы данные SFR и KFR антител оставались в общем равномерными. По сравнению с летним и зимним периодом, наличие SFR и KFR антител было весной существенно больше.

В 1986–1990 гг. исследовали на двух частных овцеводческих фермах района Страконице 341 образец сыворотки крови овец. Данные SFR были положительными в 67,1 %, KFR – 22,5 %, MPA – 0,7 %. Между обоими овцеводствами наблюдалась существенная разница в наличии SFR антител. Наличие антител в отдельные годы было в общем уравновешенным. Больше наличие антител овец, исследуемых весной и летом однако от антител, полученных осенью и зимой не отличалось.

При повторном исследовании семени 11 баранов 7 из них с SFR антителами биологическим экспериментом не удалось установить *T. gondii*.

В 1981–1990 гг. исследовали на 5 небольших фермах 54 образца кровяной сыворотки коз. Наличие SFR и KFR антител достигало 61,1 % и 21,0 %. На всех пяти фермах были у коз выявлены SFR антитела. Самое большое наличие антител относится к летнему и осеннему периоду.

Серологическим исследованием 125 овец в военном учебном лагере на территории южной Чехии были выявлены SFR антитела в 45,6 %, KFR – 12,8 %. В военном учебном лагере в западной Чехии исследовали 1 96 овец, из этого SFR позитивных достигали 73,9 %, KFR – 21,9 %.

Результаты выявили, что токсоплазмоз овец и коз на исследуемых местах нашел значительное распространение. У овец наблюдается больше на частных овцеводческих фермах чем на крупных овцеводствах. Антитела против токсоплазмоза отличаются большей устойчивостью и встречаются гораздо чаще, чем удается выявить возбудителя в тканях. Ни в одном из исследуемых овцеводческих хозяйств и стадах коз не наблюдали клинические проявления токсоплазмоза.

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