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Infestation of tracer lambs by *Fasciola hepatica* in Tunisia: determining periods for strategic anthelmintic treatments

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Summary

Ovine fasciolosis causes high morbidity and mortality rates, resulting in large economic losses to the sheep industry in northern Tunisia. The authors surveyed ovine fasciolosis *(Fasciola hepatica* infestation) in a Barbarine sheep flock in the humid region of Tunisia (Sejnane, Governorate of Bizerte) over two consecutive years, 2004 to 2005. To assess the dynamics of fluke infestation, a total of 36 tracer lambs were introduced into the flock successively, in groups of three, every two months, and their infestations were monitored by coproscopy and necropsy.

Coproscopic analysis revealed high mean percentages of infestation of 60% and 65% for flock lambs and ewes, respectively. Similarly, a high percentage of infestations was recorded in the tracer lambs (more than 70%) post mortem. Significant fluctuations in infestation dynamics were observed in the tracer lambs, and three distinct infestation periods were identified: a period of very low, non-constant infestation risk during the warm period (from July to October); a high-risk period from March to June, and a third period with a variable but constant infestation risk (from November to February).

Based on these results, the authors propose strategic flukicide treatments with triclabendazole, aimed at reducing mollusc infestation and subsequent pasture contamination by metacercariae, during two main periods: in September and in February, just before the reactivation of the molluscs from aestivation and hibernation, respectively.

Keywords

Anthelmintics – Control programme – *Fasciola hepatica* – Fasciolosis – Helminthosis – Ovine fasciolosis – Ovines – Parasites – Sheep – Tunisia.

Introduction

Sheep represent by far the most important livestock species in Tunisia. The Tunisian population is estimated at 6.5 million head of sheep (27), making Tunisia the country with the most dense sheep population in North Africa. Sheep rearing is both socially and financially important in Tunisia. For instance, it contributes 50% of the national red meat production (23). Nevertheless, several constraints remain on sheep productivity. Helminthosis causes serious economic losses in Tunisia, particularly in the humid and sub-humid zones, which are the most important areas for forage production. In these zones, fasciolosis due to *Fasciola hepatica* is a dominant and highly endemic disease which has significant impacts on animal health and financial returns. Few studies have been conducted on the prevalence of sheep infestation and on the population dynamics of the intermediate host, *Galba truncatula,* in Tunisia (3, 11). Thus, there is a need for more complete information on the infestation dynamics of *F. hepatica*, if a comprehensive strategy is to be developed for the most effective control of this helminthosis. Since the development of *F. hepatica* is closely related to fluctuations in temperature and hygrometry (15, 18, 19, 25), tracer lambs have been used in several studies to monitor infestation risks among small ruminant hosts throughout the year and to identify the best times at which to apply preventive treatments (8, 26). Unfortunately, with the exception of the work of Khallaayoune *et al.* (14) in Morocco, such studies have not been carried out in the southern Mediterranean.

In the present study, tracer lambs were used, over a period of two years, to determine the highest-risk periods for *F. hepatica* infestation of sheep when grazing in an endemic zone of northern Tunisia. The ultimate aim of this study was to develop a preventive anthelmintic programme that could be extrapolated to other regions with similar bioclimatic features in North Africa and, more generally, in the South Mediterranean Basin.

Materials and methods

Study region

This study was carried out in the marsh of Sejnane, located in the Governorate of Bizerte (37° 06' North; 09° 10' West), a swampy region characterised by hydromorphic and clay soil. This region has a Mediterranean climate with a mean annual rainfall of 1,000 mm, providing suitable conditions for the development of *G. truncatula* during the warm and wet periods of the year and, thus, the transmission of *F. hepatica*.

Animals

Sheep flock

The study was conducted in a traditional sheep flock of Barbarine sheep. The animals are extensively managed; lambs and adult animals graze together in scrub and shoal all year long. The flock was chosen partly because of the owner's willingness to cooperate and partly for its history of clinical indicator cases for gastrointestinal helminthosis (gastroenteritis, cachexia, reduced growth and mortality).

These animals are exclusively fed on the natural resources of the region; they browse on shrubs and trees of the Mediterranean bush, such as kermes oak (*Quercus coccifera*), oleaster (*Olea europaea* subsp. *europaea*), mastic (*Pistacia lentiscus*), etc., and graze on the natural herbaceous cover of different Gramineae and Leguminosae of natural pastures, fallow lands (uncultivated land during one or more years) and stubble.

The sheep are kept outdoors, except during winter, when they are housed under rudimentary shelter. Anthelmintics are exclusively used for sheep with clinical signs compatible with gastrointestinal helminthosis. The animals are treated by the owner with albendazole generics (Anthelben S, Medivet), without knowledge of the activity of the drug or the dose, and with no distinction between adults and lambs. So as not to interfere with transmission dynamics, the farmer was asked not to change any of his breeding management practices.

Tracer animals

During the survey, 36 tracer Barbarine male lambs from four to six months old and weighing, on average, 14.8 kg ± 3.6 kg were used. They were purchased over two years in groups of three from a flock located outside the endemic zones for fasciolosis in Tunisia. The absence of *Fasciola* infestations was confirmed by three coproscopic examinations at two-day intervals, using the technique described by Happich and Boray (10). The tracer animals were housed for one month in a pen at the Veterinary School of Sidi Thabet (Tunisia), where they were vaccinated against enterotoxaemia (Coglavax, CEVA santé animale), treated against scabies with diazinon (Néocidol 250, Medivet), and drenched with albendazole twice (Anthelben S, Medivet) at the conventional dose of 7.5 mg/kg, at an interval of two days.

These tracer lambs were introduced into the monitored flock in consecutive groups of three animals. Each group remained on the pasture for a period of two months; they were managed by the flock owner in the same way as the rest of the herd. At the end of the two-month period, the tracer lambs were transferred to the Veterinary School of Sidi Thabet, where they were kept indoors for three months and fed on hay and concentrate. At the end of this housing period, the lambs were slaughtered to recover flukes from their liver.

Parasitological examination

Coproscopy and haematology

Faecal and blood samples were collected every two months for two years (2004 and 2005) from 15 ewes and 15 five-to-ten-month-old lambs. These animals were randomly chosen and corresponded to 20% of the monitored flock.

Faecal samples were taken rectally, labelled and analysed within 48 h at the parasitology laboratory (Veterinary School of Sidi Thabet, Tunisia). This analysis was also performed on tracer animals at the time of their necropsies.

The technique of Happich and Boray was adopted (10) to estimate the *F. hepatica* faecal egg count (FEC). Briefly, 5 g of faeces were mixed with distilled water and filtered through a 150 µm filter. The solution was homogenised and left to form a sediment for 10 min, then the supernatant was discarded by aspiration. The pellet was mixed with one to two drops of 1% methylene blue. The solution was transferred to a gridded Petri dish to estimate the number of *F. hepatica* eggs under a stereomicroscope.

Blood samples were collected from the jugular vein in ethylene diamine tetra-acetic acid (EDTA) vacutainer tubes for haemoglobinaemia estimation with a Coulter Counter T540. The presence of anaemia was defined on the basis of haemoglobin levels below 9 g per decilitre $(dl)^{-1}$ (4).

Monitoring and necropsy of the tracer lambs

The tracer lambs were monitored for FEC and haemoglobin each month until the end of the housing period of three months at the Veterinary School of Sidi Thabet. The tracer lambs were then necropsied, the liver was removed and examined for *F. hepatica*, and the digestive tract was also examined for other helminths (data not shown). The liver was cut into thin slices (1 cm to 2 cm thick) and immersed in 9% sodium chloride solution for 3 h at 37°C. The solution was then observed for the adolescaria stage and for adult *Fasciola* parasites. The recovered flukes were preserved in 5% formalin solution.

Climatic data

The authors correlated the intensity of the infestation to climatic data from 2003 to 2005: mean, minimum and maximum temperature, rainfalls, and evaporation were gathered from the meteorological station at Sejnane (courtesy of the National Meteorology Institute, Tunisia). The moisture index, defined as the ratio of rainfall to evapo-transpiration (16), was estimated for each period.

Parasitological and epidemiological indicators and statistical analyses

The parasitological and epidemiological indicators are summarised in Table I.

When processing the results, the number of flukes recovered from the tracer lambs was expressed in relation to the corresponding grazing period. The comparison of means was conducted using the analysis of variance (ANOVA) test (24). The presence or absence of correlations between the number of *F. hepatica* in tracer animals, the FEC and haemoglobinaemia was assessed by regression analysis.

All the statistical analyses were performed with SPSS 10© for Windows© software. A probability below 0.05 was used as a cut-off value between statistically significant and non-significant results (24).

Results

Monitoring *Fasciola hepatica* using tracer animals

Twenty-five out of 36 (69.4%) tracer animals were infected with *F. hepatica*. The 11 lambs that were not infested grazed between September and October 2004 and July and October 2005. The intensity of infestation reached high values in some animals, with up to 321 *F. hepatica* adults. In 2005, tracer lambs were more heavily infested than in 2004 ($p = 0.02$) (Table II).

Table I
Epidemiological indicators of fluke infection in tracer lambs and adult animals

Epidemiological indicator	Definition	Value
Cumulative % of infected tracer lambs	100 × number of infested tracer lambs/total number of tracer lambs	69.44%
Cumulative infestation prevalence in flock lambs (%)	100 × number of flock lambs with positive coproscopy/total number of flock lambs	58.78%
Cumulative infestation prevalence in flock ewes (%)	100 × number of flock ewes with positive coproscopy/total number of flock ewes	64.57%
Mean faecal egg counts in flock lambs	Number of eggs per gram of faeces/number of examined samples	99.3
Mean faecal egg counts in flock ewes	Number of eggs per gram of faeces/number of examined samples	86.1
Individual intensity of infestation in tracer lambs	Total number of parasites/number of infested tracer lambs	55.4
Mean Hb concentration in flock lambs	Total haemoglobinaemia/number of lambs	9.16 g/dl
Mean Hb concentration in flock ewes	Total haemoglobinaemia/number of flock ewes	8.43 g/dl
Prevalence of anaemic flock lambs	Number of animals with anaemia/total number of sampled lambs	45.09
Prevalence of anaemic flock ewes	Number of animals with anaemia/total number of sampled ewes	63.16
Mean Hb values in anaemic flock lambs	Total of Hb values in anaemic flock lambs/number of flock lambs	7.27 g/dl
Mean Hb levels in anaemic flock ewes	Total of Hb values in anaemic flock ewes/number of flock ewes	7.45 g/dl
Mean Hb values in anaemic tracer lambs	Total of Hb values in anaemic tracer lambs/number of anaemic tracer lambs	7.16 g/dl
Correlation between parasite burden and Hb in tracer lambs	Parasite burden = f(Hb)	$y = 7E - 5x^2 - 0.0341x + 9.4074$ $R^2 = 0.657$

dl: decilitre g: gram
F: flock Hb: haemoglobin

Table II
Parameters of infestation of tracer lambs with *Fasciola hepatica* in Tunisian study from 2004 to 2005

Year	Number of tracer lambs	Number of infested lambs	Mean intensity of infestation (range)	Mean abundance
2004	18	14/18	35.4 (0, 246)	45.5
2005	18	11/18	75.3 (0, 321)	123.3
Total	**36**	**25/36**	**55.4**	**79.7**

The infestation intensity was highly dependent on the period during which the tracer animals were incorporated into the monitored flock (Fig. 1).

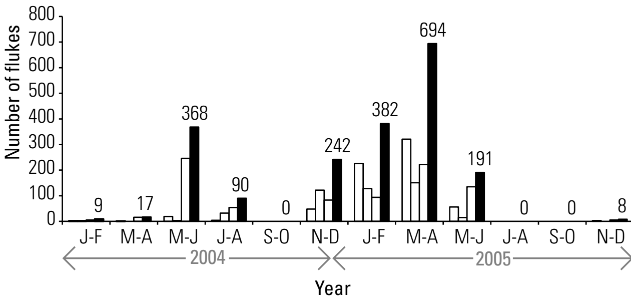

Fig. 1
Evolution of total fluke burdens in tracer lambs, according to the grazing period
Each cluster of three white bars corresponds to a group of three tracer lambs. Black bars: total parasitic charge in the three lambs

Overall, the infestation of tracer lambs increased progressively from November/December and then throughout the winter, reaching a maximum between March and April or May and June, then decreased or was even nullified in July or August. The tracer lambs did not become infested in September/October of either year (2004 or 2005). This pattern of fluke infestation was globally reproduced during the two years of the study period, although some differences and variations were recorded. These concerned: first, the infestation intensity, which was significantly higher in 2005 than in 2004 (231.4 and 80.7 flukes per tracer lamb in 2005 and 2004, respectively), and secondly, the spring peak of infestation, which occurred earlier in 2005 (March to April) than in 2004 (May to June). In 2005, this infestation peak occurred within a period of high to relatively high infestation, which extended from November to December 2004 until May to June 2005.

This pattern of infestation was associated with high moisture index values after heavy rainfalls recorded during the same period (autumn 2004 to winter/spring 2005) (Fig. 2). Each period of fluke infestation was, in most cases, preceded by or associated with both significant levels of moisture, due to heavy rainfalls, and mean temperature values above the threshold of 9°C, required for the activity of the intermediate host, *G. truncatula* (12). This relationship was particularly obvious in July to August 2004 and, to some extent, in May to June 2005, when significant parasite burdens were recorded in tracer lambs (30 and 63.7 flukes per tracer lamb, respectively). Appreciably high moisture index values had occurred in the months just prior to these periods, i.e. May to June 2004 (154 mm) and March to April 2005. The period of January to February 2005, when high infestation burdens were recorded in tracer lambs, was the only exception to this, since the average temperature during these two months was below 9°C (Fig. 3).

Most of the tracer lambs infested with *F. hepatica* (19/25) were anaemic. The mean infestation intensity was significantly higher in anaemic than in non-anaemic tracer

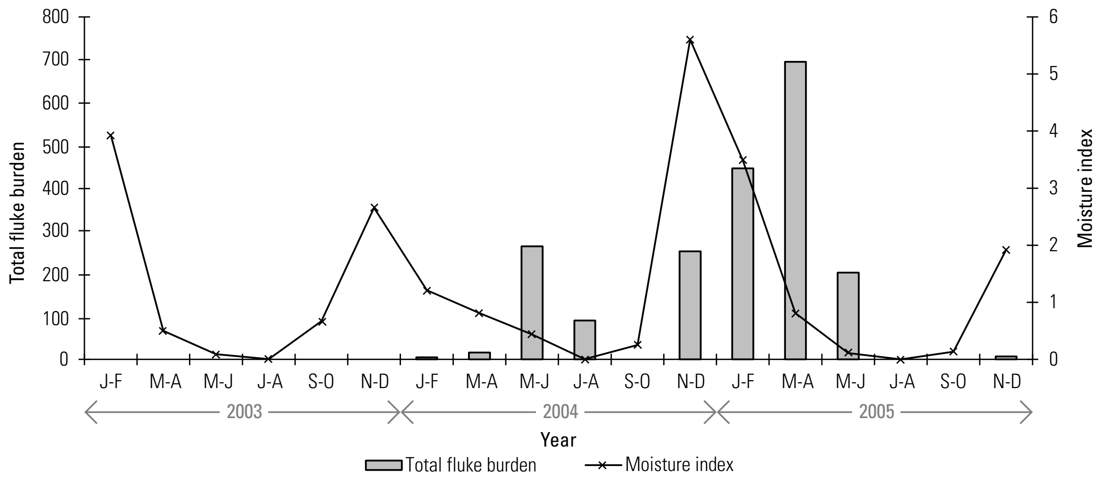

Fig. 2
Evolution of moisture index and total fluke burden in tracer lambs from 2003 to 2005

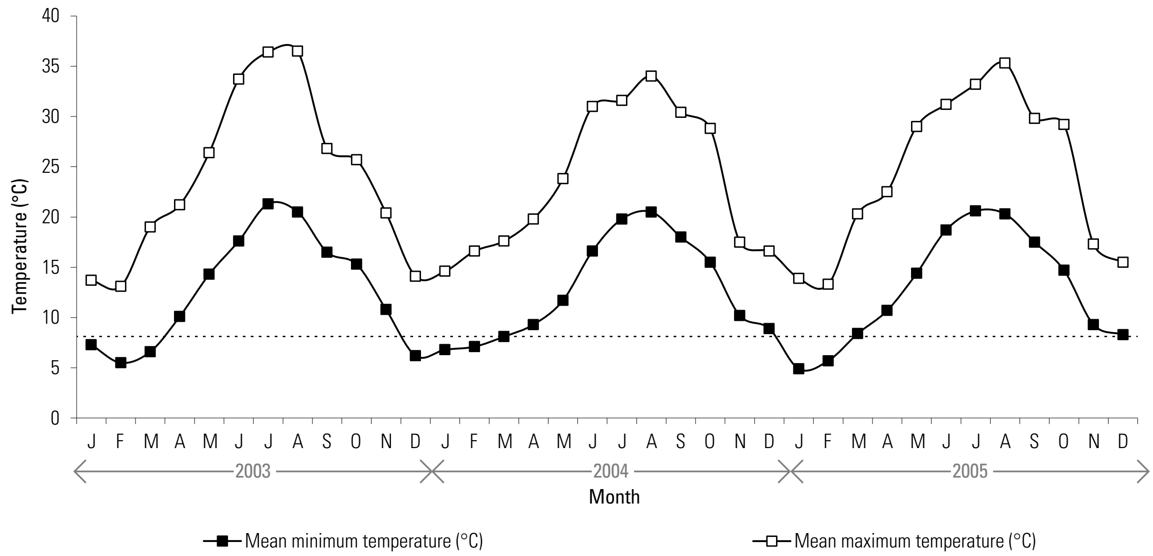

Fig. 3
Minimum and maximum mean monthly temperatures in the study region of Sejnane from 2003 to 2005
Dotted line: threshold temperature of *Galba truncatula* activity

lambs: 2.6 and 81.8 flukes, respectively (p = 0.006). Moreover, heavily infested tracer lambs developed symptoms of chronic fasciolosis. A significant positive correlation was recorded in tracer lambs between the number of flukes and the FECs ($R^2 = 0.757$, $p < 10^{-3}$). The FECs and haemoglobinaemia were also negatively correlated ($R^2 = -0.632$, $p < 10^{-3}$). A negative correlation was also observed between the number of flukes and the haemoglobinaemia ($R^2 = 0.657$, $p < 10^{-3}$) (Fig. 4). Furthermore, no negative correlations were detected in tracer lambs between haemoglobinaemia and either the number of gastrointestinal nematodes or those of *Haemonchus contortus* (data not shown). The threshold parasite burden associated with anaemia was 19 flukes; all tracer lambs harbouring more than 19 flukes were anaemic.

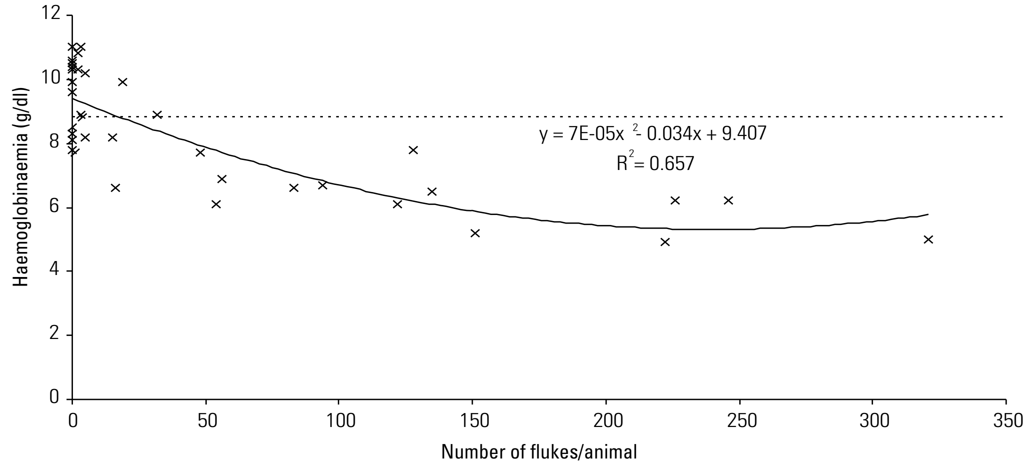

Fig. 4
Correlation between fluke burden and haemoglobinaemia in tracer lambs
Dotted line: cut-off value for anaemia

Coproscopic monitoring of the flock

The mean FEC recorded in the two cohorts of 15 ewes and 15 lambs sampled from the surveyed flock varied between 84.56 (± 182.75) and 108.70 (± 228.69) *Fasciola* eggs/g for the ewes (in 2004) and lambs (in 2005), respectively.

The two groups were found to be infested all year round. However, there were two major FEC peaks: one from July to August (2004) or May to June (2005), and a second in November to December. In ewes, the excretion peaks occurred roughly in the same period, while the spring-summer peak in lambs was less important ($p < 0.05$). The highest infestation prevalence was recorded in the summer of 2004, reaching 100% of the sampled animals (Fig. 5).

Monitoring haemoglobinaemia in the flock

Mean haemoglobinaemia values in both ewes and lambs showed a marked decrease to well below the physiological threshold during the summer (May to June or July to August) and winter seasons (from November to February) (Fig. 6), at the same time as the peak counts of *Fasciola* eggs, with values as low as 6 g/dl^{-1} (Figs 7 & 8). Interestingly, these patterns (FECs and haemoglobinaemia) could be superimposed over the periods of peak infestation in the tracer lambs. As observed in the coproscopy, the periods when haemoglobinaemia fell were quickly followed by re-establishment towards normal values, due to treatment of the diseased animals by the owner with albendazole. The tracer lambs, however, were not targeted

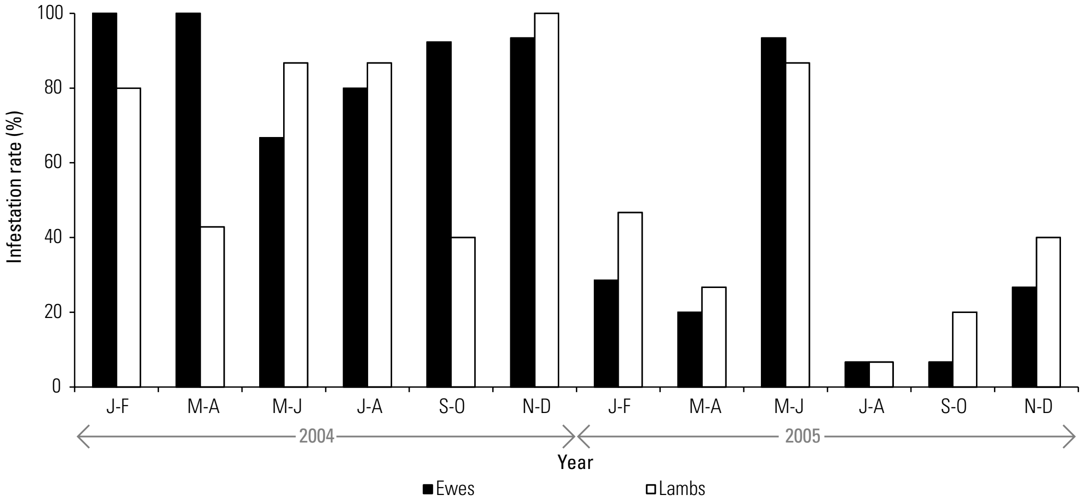

Fig. 5
Infestation rate in the two age groups, ewes and lambs, of the surveyed flock

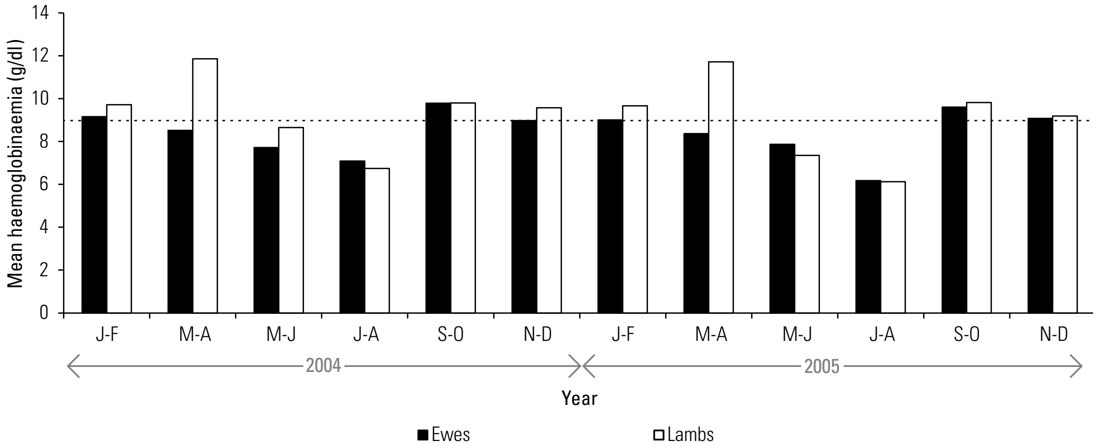

Fig. 6
Mean haemoglobinaemia in the two cohorts of 15 lambs and 15 ewes sampled from the surveyed flock
Dotted line: cut-off value for anaemia

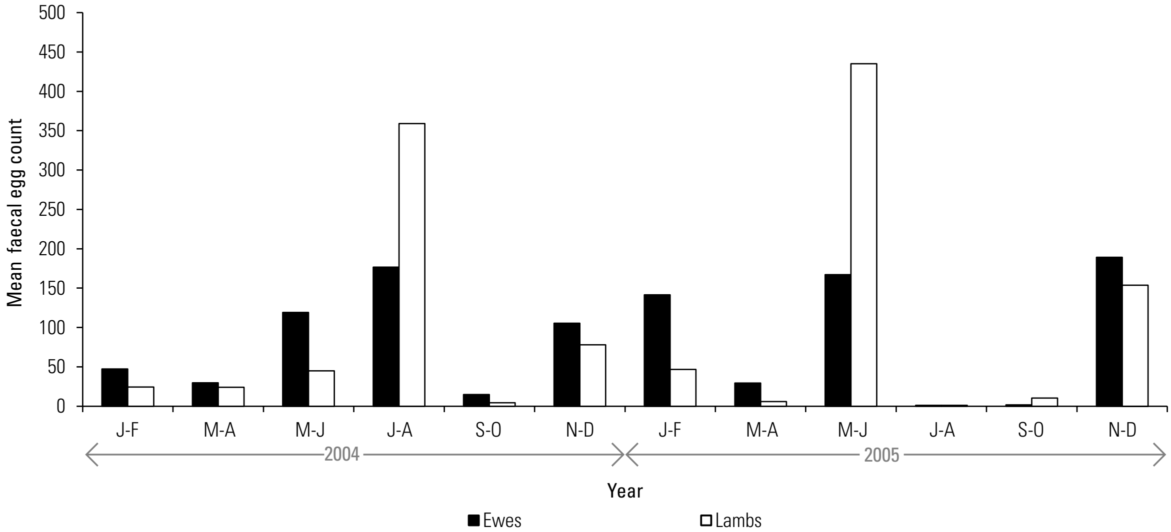

Fig. 7
Mean faecal egg counts of *Fasciola hepatica* in the two cohorts of 15 lambs and 15 ewes sampled from the surveyed flock

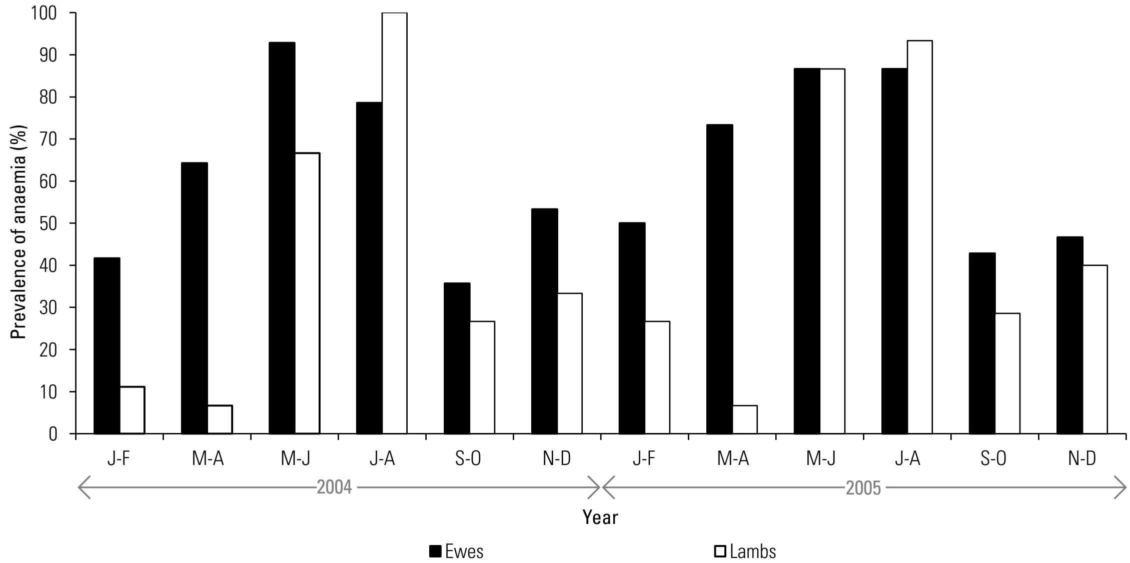

Fig. 8
Prevalence of anaemic animals in the two cohorts of 15 lambs and 15 ewes sampled from the surveyed flock

by these treatments. There was no difference between the mean haemoglobinaemia values in lambs and ewes (p = 0.227). However, the prevalence of anaemic individuals was significantly higher among ewes. Indeed, the cumulative prevalence of anaemia reached 45.09% and 63.16% in lambs and ewes, respectively (p = 0.0007).

Discussion

The Sejnane region (Governorate of Bizerte) is well known in Tunisia as the most endemic zone for sheep fasciolosis in the country (11). The soil profile, together with the hydrological and topographic characteristics of this region, is particularly favourable for the development of the amphibious gastropod *G. truncatula,* the intermediate host of *F. hepatica* (11).

In this survey, the authors introduced successive groups of tracer lambs onto these pastures for a period of two months, to determine various parasite burdens during different grazing periods. In conjunction with this, they also measured the flock's parasitological and morbidity (haemoglobinaemia) indicators. This approach was an attempt to link pasture infestation to the effects of fluke infestation on the health of the surveyed herd. Taking into account that fasciolosis is usually expressed in Tunisia as a chronic, long-lasting form of the disease, the tracer lambs were kept indoors for three months after the grazing period, to ensure that all acquired parasites could develop into mature adults.

During the two successive study years (2004 and 2005), a high prevalence of fluke infestation was recorded in both the flock's ewes and lambs, via coproscopy, and in the tracer lambs via necropsy (Table II) (Fig. 5). These results were significantly higher than those of Jemli *et al.* (11), who recorded a lower prevalence of infestation (11.5%) in sheep flocks from the same zone, although these authors used a different coprological technique. In an endemic zone of the High or Middle Atlas in Morocco, Khallaayoune *et al.* (14) reported similar prevalences of *F. hepatica*, varying between 20.5% and 67.4%.

The parasite burdens recorded in the present study varied between 0 and 321 flukes per tracer lamb. Overall, these results were quite similar to those reported in Morocco by Khallaayoune *et al.* (14) (range 0 to 345 flukes).

The seasonal dynamics of *F. hepatica* infestation are conditioned by climatic conditions and whether these are suitable for lymnaeid snails, the evolution of the fluke eggs into miracidia and the development and survival of metacercariae on pastures. Reproduction of the lymnaeid snail requires a mean temperature above 10°C, whereas the development and release of metacercariae is possible when the temperature is higher than 16°C (1). At optimal temperatures (above 20°C), the minimum duration of the outdoor stage of *F. hepatica* is estimated at six to seven weeks (28) but can sometimes reach three months (9) at lower temperatures. The cycle is, however, interrupted below 9°C (12). Metacercariae maintained in the laboratory at 10°C keep their infectivity for 31 and 122 days at a relative humidity of 75%–80% and 90%, respectively (5).

Also in the Sejnane region, Jemli *et al.* (11) demonstrated the presence of two lymnaeid snail activity peaks: one in spring (April) and another in autumn (November). These periods provided good conditions for a longer lifespan of metacercariae in pastures.

The authors used the moisture index in this study, since this indicator gives a good estimate of the proportion of rainwater that remains available in the ground for the development of the amphibian lymnaeid snails. Indeed, this index has been shown to be a reliable indicator for analysing the activity of *Anopheles* sp. mosquitoes (16).

Three constant periods could be identified, based on the results recorded in tracer lambs and taking into account the climatic conditions, average monthly temperature and, in particular, the moisture index.

The first period occurred from September to October. The tracer lambs were not infested during these months in either 2004 or 2005. This period was consistently preceded by at least one or two prior consecutive periods of zero or almost zero moisture, according to the index (rainfall lower than evapo-transpiration: 96 mm in 2004 and 51 mm in 2005), and high temperatures (from 28.8°C to 34.4°C in 2004 and 29.2°C to 35.3°C in 2005). These climatic conditions, characteristic of the Mediterranean summer and early autumn, cause the interruption of *Galba* activity, as also reported by Jemli *et al.* in the same region (11). In addition, they provide unfavourable conditions for the survival of the metacercariae, which are very sensitive to dry and hot conditions (2, 18, 20). Similar results have been reported in the United States (8) and in Morocco (14).

A second period of constant but variable infestation tends to extend from November to February. High parasite burdens were recorded during these months in 2004 and low burdens in 2005 (Fig. 1). During this period, the moisture index reached its highest annual values, whereas temperature decreased to below the threshold required for *Galba* activity and the development of *Fasciola* eggs from December to February. Accordingly, the infestation recorded during this second period was due to transhibernating metacercariae from snails active during the autumn. Indeed, metacercariae maintained in the laboratory at 10°C keep their infectivity for up to 122 days at a relative humidity of 90% (5).

The magnitude of fluke burdens during this second period, particularly noticeable from November/December 2004 to January/February 2005, probably depends on two distinct conditions. The first is the occurrence of a relatively high moisture index in late spring and early summer of the previous year, as observed in May and June 2004, which creates good conditions for increasing the summer snail population, since a low number of infested molluscs active in summer can lead to a high level of pasture contamination (6, 15). The second factor is a concomitant high moisture index that favours the extension of the *Galba* habitat and the dispersion of metacercariae, after the dry period of the year. This extension of the marshy area, in addition to enlarging the *Galba* microhabitat, probably also has important implications for the dispersion of metacercariae on usually grazed parts of the lowlands.

A third period of constant infestation peaks between March/April and May/June. This period coincides with the spring activity of *Galba* (11), when significant moisture index values are associated with mean temperatures above 10°C. The importance of the infestation peaks recorded in the spring of both 2004 and 2005, and their chronology, appears to depend more on the moisture index and the average temperatures recorded during the previous months than on the water balance registered during the spring period itself. Indeed, in spite of large differences in parasite burdens in tracer lambs, the springs of 2004 and 2005 had almost similar moisture indices, particularly from March to

April. The comparatively high, early infestation peak observed from March to April 2005 was associated with a wetter autumn and winter in 2004 to 2005, as indicated by a moisture index that was almost twice as high, when compared to conditions in 2003–2004 and 2005. These conditions probably increased the population of hibernating *Galba,* as well as increasing pasture contamination by transhibernating metacercariae, and resulted in extending the snail habitat after its reactivation in the spring. In addition, the relatively higher average temperatures registered in March and April 2005 (Fig. 3) must also be considered.

In addition to the three constant periods described above, a fourth non-constant infestation period from July to August – two months characterised by a zero moisture index – was identified. The presence of infestation during this period is associated with a significant moisture index in May and June, as noted in 2004, for example. In contrast, the absence of infestation in July and August seems to require more than two months of drought (as in 2005), probably to ensure desiccation of the snails' habitat zones reached by the animals during grazing and the destruction of the metacercariae dispersed in the environment. The longer lifespan of metacercariae in humid microhabitats (for example, in persistent grass around wet parts of the pasture) should probably also be considered as an additional risk factor for infestation (13, 29), particularly during the hot season which begins in May and June. It is, therefore, not surprising that the individual parasite burdens recorded in tracer lambs during the summer months (May to August) showed the highest variation among individuals. On the other hand, less variable individual parasite burdens occurred at periods of higher infestation pressure, due to more homogeneous distribution and better survival of metacercariae on pastures (Fig. 1). Taken all together, these observations indicate that the use of sentinel animals enables a fairly reliable evaluation of the risks of fluke infestation, at least under the conditions observed in the present study. This conclusion is supported by the correlation found between the infestation burdens recorded in tracer lambs and the FECs and haemoglobinaemia values measured in flock ewes and lambs. Indeed, *F. hepatica* appears to be the major cause of anaemia recorded in the majority of sampled ewes and lambs from the surveyed flock, as particularly indicated by:

– the presence of a strong correlation between haemoglobinaemia values and fluke burdens in tracer lambs ($R^2 = 0.657$, $p < 0.001$), and the absence of a correlation with gastrointestinal nematodes in the same animals (data not shown) ($R^2 = 0.004$, $p = 0.730$)

– the recording of severe anaemia in the lambs and ewes of the flock in conjunction with FEC peaks for *Fasciola* eggs

– the occurrence of the lowest haemoglobinaemia values and the highest prevalence of anaemia (Figs 6 & 8) in ewes and lambs during high-risk periods for chronic forms of fasciolosis, with regard to parasite burdens and haemoglobinaemia values recorded in the tracer lambs.

The prevalence of anaemia in the flock's ewes and lambs started to increase from autumn and peaked in summer, decreasing to its lowest levels in September and October. This pattern could be explained by the infestation charges of the tracer lambs if considering the chronology of fluke entry into the biliary ducts. Furthermore, the highest prevalence of anaemia (98.83%), as well as the lowest haemoglobinaemia values, was recorded in July and August, two to three months after the infestation peak in tracer lambs between March and June. A good match was achieved by superimposing the data recorded from the tracer lambs onto the data from the flock ewes and lambs, and this was supported by an obvious relationship between the number of parasites in tracer lambs and the FEC in adult animals ($R^2 = 0.757$; $p < 0.001$) A similar correlation was also reported in goats in Morocco (13). The distinct falls recorded after FEC peaks were always associated, particularly in the ewes, with increased haemoglobinaemia (Figs 7 & 8). This particular evolution could be explained by the use of curative anthelmintic treatments, carried out by the sheep owner, when the animals were showing obvious clinical signs of fasciolosis.

In light of the results of this study, fasciolosis appears to be a major parasitic disease in the study region. Taking into account both the parasite burdens and haemoglobinaemia values described above, this helminthosis must have severe economic consequences. Indeed, livers condemned during the summer for fasciolosis represent between 45.5% and 80% of livers destroyed each year at the Sejnane slaughterhouse (3, 11).

The curative administration of anthelmintics performed by the sheep owner over the two years of the study was not effective in controlling fasciolosis in the herd. This demonstrates the necessity for a comprehensive control programme against this important parasitosis. In Ireland, Parr and Gray (22) confirmed the effectiveness of a strategic dosing scheme of sheep with triclabendazole, designed to minimise contamination with *F. hepatica* eggs during the active period of the lymnaeid intermediate host. This approach is based principally on treating sheep two to three times during spring and summer, at approximately eight-to-ten-week intervals.

Based on the results of the present study, the authors propose a strategic dosing scheme consisting of two anthelmintic treatments against fasciolosis, to limit the coinciding period between active molluscs and developing *F. hepatica* eggs: a first treatment in September, just before the emergence of *Galba* from aestivation, and a second from mid-to-late February, just before reactivation of these molluscs from hibernation. In this context, and due to its strong activity on the early immature stages of the fluke, triclabendazole is expected to achieve better control of infestation, due to a longer period of suppression of fluke egg output in sheep during the active phases of the lymnaeid intermediate host (7, 21, 22). However, the use of other flukicides, which are less active on the immature stages of *F. hepatica*, might require a third treatment between March and April, the period when the intermediate hosts are at their most active.Given the use of common pastures and range lands in the study region, the strategic dosing scheme described above should be applied collectively to the whole sheep population of the endemic region to ensure effective control of fluke infestations. However, such use of flukicides two to three times a year should be accompanied by practices aimed at reducing the risk of emergence of drug-resistant *F. hepatica*, since this has previously been documented for triclabendazole (17).

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

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L'infestation d'agneaux traceurs par *Fasciola* hepatica en Tunisie : détermination des périodes de traitements anthelminthiques stratégiques

H. Akkari, M. Gharbi & M.A. Darghouth

Résumé

La fasciolose est à l'origine d'une morbidité et d'une mortalité élevées entraînant de lourdes pertes économiques pour le secteur de l'élevage ovin du nord de la Tunisie. Les auteurs présentent les résultats d'une étude sur la fasciolose ovine (infestation par *Fasciola hepatica*) conduite pendant deux années consécutives (2004 et 2005) dans un troupeau ovin de race Barbarine situé dans une région humide de la Tunisie (plus précisément à Sejnane, localité située dans le gouvernorat de Bizerte). Afin d'évaluer la cinétique de l'infestation par la douve du foie, 36 agneaux traceurs ont été progressivement introduits dans le troupeau, à raison d'un groupe de trois agneaux tous les deux mois ; l'existence d'une éventuelle infestation a été recherchée par coproscopie et lors de l'autopsie.

L'analyse coproscopique a révélé des taux moyens d'infestation élevés, allant de 60 % chez les agneaux à 65 % chez les brebis. De même, un taux d'infestation élevé a été constaté chez les agneaux traceurs lors des examens post mortem (plus de 70 %). La cinétique d'infestation a présenté des variations significatives chez les agneaux traceurs, avec trois périodes distinctes: une période d'infestation très faible et par intermittences, pendant la période chaude (de juillet à octobre) ; une période à haut risque, allant de mars à juin ; et une troisième période caractérisée par un risque d'infestation variable mais ininterrompu (de novembre à février).

En se basant sur ces résultats, les auteurs préconisent un traitement douvicide au triclabendazole, dans le but de limiter l'infestation des mollusques et la contamination subséquente des prairies par les métacercaires, à administrer au cours de deux périodes distinctes : en septembre et en février, c'est-à-dire juste avant la réactivation des mollusques sortant d'estivation et d'hivernation.

Mots-clés
Anthelminthique – *Fasciola hepatica* – Fasciolose – Fasciolose ovine – Helminthose – Mouton – Ovin – Parasite – Programme de lutte – Tunisie.

■

Infestación de corderos trazadores por *Fasciola hepatica* en Túnez: determinación de periodos para administrar tratamientos antihelmínticos estratégicos

H. Akkari, M. Gharbi & M.A. Darghouth

Resumen
La fasciolosis ovina se acompaña de elevadas tasas de morbilidad y mortalidad y causa por ello grandes pérdidas económicas al sector ovino en el norte de Túnez. Durante dos años consecutivos, 2004 y 2005, los autores estudiaron la fasciolosis ovina (infestación por *Fasciola hepatica*) en un rebaño de ovejas barbarinas de la región húmeda de Túnez (Sejnane, provincia de Bizerte). Para determinar la dinámica de la infestación se introdujeron en el rebaño sucesivamente, una vez cada dos meses por grupos de tres, un total de 36 corderos trazadores, cuya posterior infestación se controló por coproscopia y necropsia.

Los análisis coproscópicos revelaron altos porcentajes medianos de infestación, de un 60% y un 65% en los machos y las hembras del rebaño, respectivamente. Los análisis *post mortem* pusieron también de manifiesto un alto porcentaje en los corderos trazadores (más del 70%), cuya dinámica de infestación mostraba importantes fluctuaciones, que sirvieron para distinguir claramente tres periodos distintos: una fase de riesgo de infestación muy bajo e inconstante durante la estación cálida (de julio a octubre); un periodo de alto riesgo de marzo a junio; y una tercera fase de riesgo variable pero constante (de noviembre a febrero).

Atendiendo a esos resultados, los autores proponen la administración estratégica de tratamientos vermífugos con triclabendazol destinados a reducir la infestación de moluscos, y la subsiguiente contaminación de los pastos por metacercarias, en dos periodos básicos: septiembre y febrero, justo antes de la reactivación de los moluscos tras los letargos estival e invernal, respectivamente.

Palabras clave
Antihelmínticos – *Fasciola hepatica* – Fasciolosis – Helmintiasis – Fasciolosis ovina – Oveja – Ovinos – Parásitos – Programa de control – Túnez.

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