# Clinico-pathological studies in cattle experimentally infected with *Taenia saginata* eggs

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# ABSTRACT

Calves 1-2 months old were experimentally infected with eggs of Taenia saginata and clinical and haematological deviations, development and distribution of cysticerci and pathological changes were recorded. The calves infected with 5 000, 10 000 or 50 000 eggs showed an increase in pulse and respiratory rates. The animals that received 50 000 eggs had significantly increased pulse (p < 0.05) and respiratory rates (p < 0.005). The symptoms were more severe in young, 30-day-old calves infected with 50 000 eggs. Haemoglobin concentration, haematocrit values and red blood cell count decreased, but white blood cell count increased slightly. Lymphocytes and eosinophils also increased up to 88 % and 14 %(p < 0.05), respectively. Most of the cysticerci were not fully formed 1 month post-infection, but at 2 months the cysts were fully mature and at 4 months, some cysts had degenerated. There was no uniform pattern of distribution of cysticerci in the body of infected calves, but the most commonly affected sites were masseter and heart muscles, followed by diaphragm, tongue and other skeletal muscles. The maximum concentration of 8-14 cysticerci per 10 g of tissue was recorded in masseter muscles and heart. The affected parts revealed tissue reactions that included pressure atrophy, necrosis and fibrosis. Microscopically, the lesions comprised infiltration with lymphocytes, plasma cells, eosinophils and macrophages, fibrosis, necrosis and calcification. The tissue reaction was severe in calves infected with 50 000 eggs. The severity of clinical signs, haematological and pathological changes depended mostly on the age of the animals and dose of infection

**Key words**: clinical and haematological deviations, *Cysticercus bovis*, pathology, *Taenia saginata*.

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# INTRODUCTION

Taenia saginata cysticercosis in cattle is a serious veterinary and public health problem worldwide<sup>6-8</sup>. Moderate to high prevalence of this parasite has previously been observed in cattle in Fars Province, Iran<sup>3,11</sup>. High percentages of calves are susceptible to *T. saginata* infection shortly after birth, while artificial infection becomes progressively more difficult and finally impossible with increasing age<sup>5,16</sup>. Variable tissue reactions, clinical and haematological findings have been reported previously<sup>13,14,17</sup>. Owing to the lack of uniformity in the earlier reports and considering the seriousness of the problem in Iran, calves of different age groups were experimentally infected with various doses of T. saginata eggs to observe clinico-haematological deviations, distribution pattern of the cysticerci and tissue reactions at different stages of infection.

# MATERIALS AND METHODS

Eggs and gravid segments of T. saginata were obtained from infected human patients. The eggs were repeatedly washed with physiological saline and used as fresh as possible. They were counted in McMaster chambers and suitable dilutions were prepared in physiological saline to infect the clean calves, which were obtained from cows that were treated and maintained under parasite-free conditions. These calves were maintained indoors under similar conditions and infected orally with different doses of eggs (Table 1). The calves were kept under careful observation. Their clinical signs, including body temperature, pulse rate, respiration rate and any other clinical manifestations, were recorded before infection and then weekly post-infection until the end of the experiment. Blood samples were examined on Day 0 and weekly thereafter.

One animal from each group was necropsied 1, 2, and 4 months postinfection and all body parts were examined for the presence of cysticerci and gross lesions. Aliquots of muscular tissues were digested in pepsin and the cysticerci in different stages of development were collected and counted per 10 g of muscle. Muscle samples from the infected body parts were also examined randomly for the presence of cysticerci and the cysts thus recovered were counted. The stages of development of the cysts and their dimensions were recorded. Suitable tissue samples were also collected in formol saline and processed by routine histological techniques to examine the microscopical lesions<sup>11</sup>. The data from infected and uninfected control animals were compared statistically using paired t-test and analysis of variance. These analyses were carried out using the Statistical Package for Social Sciences (SPSS). The statistical tests were expressed as significant at the 95 % confidence level.

#### RESULTS

#### **Clinical signs**

Deviations in pulse and respiratory rates and in the temperature of calves infected with different doses of *T. saginata* eggs are presented in Table 2 and Figs 1–3.

The pulse rate of calves infected with different doses deviated significantly at different intervals. Calves infected with 5000 eggs showed an average increase in pulse rate from 54 to 88.5 (p < 0.05) in the 3rd week of infection, whereas during this period in the group of calves infected with 10 000 eggs, the increase was from 64.0 to 104.0 (p < 0.05). Calves infected with 50 000 eggs showed a significant increase in pulse rate from 55 to 102 (p <0.05) in the 4th week of infection. After the 4th week, the pulse rate in all infected calves started decreasing and ranged from 60.5 to 66.0 in the 16th week post-infection, which was similar to the controls.

The body temperature of the calves remained normal until the 2nd week of

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Table 1: Infection schedule for bull calves infected orally with eggs of Taenia saginata.

Group number	Number of calves in each group	Age of calves (months)	Number of eggs/calf			
1	3	1–2	5 000			
2	3	1–2	10 000			
3	3	1–2	50 000			
4	2	1–2	Uninfected control			

Table 2: Deviations in pulse and respiration rates, and body temperature of infected and control calves.

Group number	Infection dose		a	
	(number of Taenia saginata eggs)	Pulse	Respiratory rate	Body temperature (°C)
1	5 000	54–88.5	16–28	38.50–39.35
2	10 000	64–104	16–32	38.30-39.40
3	50 000	55-102**	14–36***	38.30-40.40**
4.	Nil	62–66	17–28	38.50–39.20

<sup>a</sup>Recorded morning and evening daily.

\*\*Significant at p < 0.05.

\*\*\*Significant at p < 0.005.

infection, when it increased from 38.3 to 39.4 °C in calves infected with 10 000 eggs. In calves infected with 5000 and 50 000 eggs, the temperature reached 39.35 and 40.40 °C, respectively, in the 4th week of infection, compared to their respective body temperature of 38.5 °C and 38.3 °C pre-infection. The respiratory rate also showed a slight increase, from 16.0 to 21.5/min in the 2nd week of infection, but in the 4th week post-infection, it increased significantly to 36.0/min (p <0.005) in 30-day-old calves infected with 50 000 eggs. These calves were severely affected and showed acute clinical signs, such as respiratory distress, in the 3rd and 4th week of infection, and 1 calf developed tachycardia.

#### Haematology

The haematological values of calves infected with various doses of T. saginata eggs are presented in Table 3. The haemoglobin (Hb) contents, red blood cell count (RBC) and haematocrit (PCV) decreased between the 8th and 10th week of infection in animals infected with 5000, 10 000 and 50 000 eggs. However, the decrease in Hb content was significant (p < 0.05) during 8th to 10th week of infection in calves infected with 50 000 eggs and a decrease to 9.6 g/d $\ell$  was observed in the 10th week of infection. RBC count ranged between 6.1 and  $7.9 \times 10^6$ /mm<sup>3</sup> during the 8th and 10th week of infection as against the control values of  $9.2-9.4 \times 10^{6}$ /mm<sup>3</sup>. The decrease in RBC count started in the 4th week and continued until the 10th week of infection. The RBC count in calves infected with 50 000 eggs decreased from  $9.5 \times 10^{6}$ /mm<sup>3</sup> to  $6.1 \times 10^{6}$ /mm<sup>3</sup> in the



Fig. 1: Pulse rate of calves infected with Taenia saginata eggs.

10th week post-infection. The minimum RBC count in calves infected with 5000 and 10 000 eggs was 7.9 and  $7.8 \times 10^6$ /mm<sup>3</sup> respectively. PCV decreased to 28 % in the 10th week post-infection in calves infected with 50 000 eggs compared to the control values of 48–50 %. The calves infected with 5000 and 10 000 eggs showed respective PCV values of 35 % and 31 % in the 9th and 8th week.

Total WBC count increased slightly in all calves infected with 10 000 and 50 000 eggs. The maximum increase was in the 3rd week of infection *i.e.*  $9400/\text{mm}^3$  in

calves infected with 10 000 eggs compared to 7300/mm<sup>3</sup> in controls. Differential leukocyte count (DLC) revealed an increase in lymphocyte and eosinophil counts in all calves infected with 5000, 10 000, and 50 000 eggs of *T. saginata*. Lymphocytes increased up to 88 % in calves infected with 50 000 eggs in the 4th week post-infection compared to control values of 56 %. In calves infected with 5000 and 10 000 eggs, the maximum numbers were 76.0 and 80.0 %, respectively, in the 4th week. The number of eosinophils in this infection increased



Fig. 2: Respiratory rate of calves infected with Taenia saginata eggs.



Fig. 3: Body temperature of calves infected with Taenia saginata eggs.

significantly (p < 0.05) to 14.0 % in the 5th week of infection in calves infected with 10 000 eggs compared to the control values of 0–1 %. The number of other leukocytes remained almost unaltered in all calves during the experiment.

#### **Development of cysticerci**

At 1 month post-infection, few cysticerci were fully formed, measuring from  $2-7 \times 1-3$  mm; however, the cyst walls were fragile and in most cysts, the scolex was not clear. At 2 months post-infection, the

size of cysticerci was larger and they measured 3–8 × 2–4 mm. The cysts were well developed, with a fairly strong wall, and the scolex was clearly visible. Most cysticerci were mature at this stage. At 4 months post-infection, the cysticerci measured 4–10 × 2–5 mm. The cysts were fully mature, containing fluid and a welldeveloped scolex. However, some of the cysts at this stage were degenerate and contained greenish material. A few cysts in muscles were hard and calcified. The cysticerci attained a larger size and developed more rapidly in calves infected with 5000 eggs at the age of 30 days than in those infected with higher doses (10 000 and 50 000 eggs) at 2 months of age.

# Distribution of cysticerci and intensity of infection

There was no uniform pattern of distribution of cysticerci in various body parts of all calves infected with different doses of eggs of T. saginata (Table 4). However, the most seriously affected body parts in most cases, irrespective of dose and time interval, were masseter muscles and heart (Figs 4, 5) followed by diaphragm (Fig. 6), intercostal muscles, tongue, muscles of forelimbs and hind limbs, abdominal muscles and oesophagus. In 1 calf infected with 10 000 eggs, a few cysts were observed in the liver. In another calf infected with 50 000 eggs, 6 cysts were observed in the lungs, 1 each in the kidneys, outer surface of the rumen and male genitalia. However, no cysts were observed in the brain of any of the calves. The number of cysticerci per 10 g of tissue revealed that masseter muscles and heart had the highest concentration, i.e. 8-14 cysts per 10 g of tissue (means 8.6 and 8.2 cysts respectively), followed by diaphragm 1.5-13 cysts (mean 5.0), while intercostal, abdominal and fore and hind limb muscles and tongue had 0.6-8.0 cysts per 10 g (Table 4).

# Pathology

#### Gross lesions

At 1 month post-infection, macroscopic examination revealed cysticerci of different sizes embedded in the tissues of the various organs and different muscles of all calves. The cysts in these organs were lying in different planes, causing slight to moderate damage in different locations. Some of the cysts were not fully formed and none was mature. Heart, tongue and oesophagus showed no significant lesions except for pressure atrophy where large numbers of cysts were aggregated. In the heart, the cysts were mostly embedded in the adipose connective tissue and partly in the myocardium. The skeletal muscles were most affected and the cysts were embedded in the tissue, causing local tissue reaction. At the site of heavy infection, the normal texture of the muscles was seriously affected. In the diaphragm, the cysticerci were mostly present on the outer surface, causing damage to the underlying tissue. The organs with 1 or 2 cysts did not show any lesions.

At 2 months post-infection, many cysts were mature and larger in size in different locations in all animals irrespective of

#### Table 3: Haematological values of infected and control calves.

Group	Infection dose	Haematological values (range and mean)											
	(Number of	RBC	<b>WBC</b> (×10 <sup>3</sup> )	<b>Нb</b> (g/d <i>l</i> )	PCV	Differential leucocyte count (%)							
	Taenia saginata eggs)	(×10 <sup>6</sup> )			(%)	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil			
		$\leftarrow$		$\leftarrow$	$\leftarrow$								
1	5000	7.9–9.8	6.6-7.9	11.6–13.0	35–49	27–29	46-76	5–7	2–8	-			
		(8.6)	(6.8)	(12.8)	(45.0)	(27.8)	(67.3)	(5.5)	(2.9)				
		$\leftarrow$		$\leftarrow$	$\leftarrow$								
2	10 000	7.8–9.6	7.1–9.4	10.3–14.6	31–44	24–26	52-80	8–10	1–14	0–1			
		(8.7)	(8.5)	(12.0)	(36.0)	(25.2)	(72.1)	(8.6)	(6.5)*	(0.02)			
		$\leftarrow$		$\leftarrow$	$\leftarrow$			$\leftarrow$					
3	50 000	6.1–9.5	7.3–9.0	9.6-14.0	28–46	25–30	55–88	5–4	1–9	-			
		(7.8)	(8.4)	(10.8)*	(33.0)	(28.1)	(76.2)	(4.1)**	(4.8)**				
		$\rightarrow$		$\rightarrow$	$\rightarrow$								
4	Uninfected controls	9.2-9.4	7.3-8.7	14.0–15.0	48–50	24–30	50–56	5–6	0–1	-			
		(9.3)	(7.6)	(14.4)	(49.0)	(27.5)	(53.5)	(5.4)	(0.2)				

\*Significant at p < 0.05. \*\*Significant at p < 0.02.

#### Table 4: Intensity of infection with cysticerci in calves experimentally infected with Taenia saginata eggs.

Grou	o Infection	Post-infection interval (months)	Number of cycst per 10 g of tissue (mean)											Size of cysticerci			
	dose (number of) eggs)		Tongue	Oesoph.	Rumen	Heart	Lungs	Liver	Diaphr.	Fore- limbs	Hind limbs	Masseter muscles	Intercostal muscles	Abdominal muscles	Kidneys	Male genitalia	Range (L × W) <sup>a</sup> and (means) (mm)
1		1	1 <sup>b</sup>	2 <sup>b</sup>	-	0.3	-	2 <sup>b</sup>	1.5	0.8	0.8	1	1	1	-	-	$2-7 \times 1-3$ (3.5) (2.0)
	5000	2	0.6	-	-	4	-	-	3	1.1	0.6	1.6	1.6	1.6	-	-	3–8 × 1.5–3 (5.5) (2.5)
		4	-	-	-	9 <sup>b</sup>	-	-	2 <sup>b</sup>	-	1	1	1 <sup>b</sup>	-	-	-	$4-10 \times 2.5-4.5$ (8.5) (3.5)
2		1	3.3	-	-	2.6	-	-	3.3	2	3	6.6	2	2	-	-	$2-4 \times 1-2$ (3.0) (1.5)
	10 000	2	<b>1</b> ⁵	1 <sup>b</sup>	-	2.3	-	1.6	2.6	1.1	0.6	0.6	0.6	2	-	-	$3-7 \times 2-4$ (5.3) (2.3)
		4	1 <sup>⊳</sup>	4 <sup>b</sup>	-	6.6	0.3	2 <sup>b</sup>	3.3	1.1	2.3	2.6	0.6	1.6	-	-	$4-8 \times 2-5$ (6.8) (3.0)
3		1	7.6	-	-	7	-	4.1	13	7.8	6.6	8.6	4	4.3	-	-	$2-5 \times 1-3$ (3) (2)
	50 000	2	8.0 <sup>b</sup>	3 <sup>b</sup>	-	1	-	-	9.3	4.6	6.0	4	8	5	-	-	$3-7 \times 2-4$ (4.0) (2.4)
		4	3	1 <sup>b</sup>	1 <sup>b</sup>	8	6 <sup>b</sup>	-	5.3	3.8	4	14	3.3	3.3	1⁵	1 <sup>b</sup>	$4-9 \times 2-5$ (8.0) (3.0)
4 U	ninfected con	trol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

<sup>a</sup>L = length; W = width.

<sup>b</sup>Number of cysticerci in entire organ.

dose of infection. The lesions were almost similar to those at 1 month post-infection but were a little more chronic. In places, slight fibrosis was observed, which was more perceptible in skeletal muscles.

At 4 months post-infection the cysts were fully mature. The scolex was easily seen through the cyst wall in situ. However, some of the cysts were degenerate and calcified. On sectioning, they revealed hard, greenish material. The tissue around the cysts revealed fibrosis and calcification.

#### Microscopic lesions

The calves infected with 5000 eggs of T. saginata at 1 month post-infection

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revealed many cysticerci with the cyst wall, suckers and scolex in the process of development. The cyst wall was very thin and weak. Development of cysts in different locations showed some variation, as the cysts in the hind limbs developed better, with a well-developed cyst wall and prominent scolex. Necrosis of the tissue was observed at the site of attachment and numerous inflammatory cells infiltrated this area. The predominant cells were lymphocytes, plasma cells, eosinophils and macrophages. In places, fibroblasts were proliferating to form collagen fibres around the cyst. At 2 months post-infection, the cysticerci were fully formed and infiltration was more

pronounced (Figs 7, 8). Tissue necrosis, particularly in the heart, was reduced and the inflammatory cells, especially macrophages, were more numerous compared to 1 month post-infection. Fibroblastic proliferation and granulation-tissue formation was fairly prominent at this stage. At 4 months post-infection, welldeveloped, large cysts were seen in different locations. Some of the cysts were hard, degenerate, calcified and surrounded by thick layer of fibrous connective tissue. The inflammatory reaction was intense and included infiltration of lymphocytes, plasma cells, eosinophils and large number of macrophages (Fig. 9). There was fibroblastic proliferation



Fig. 4: Severe infection of masseter muscles with cysticerci in a calf that received 50 000 eggs of *Taenia saginata* (4 months post-infection).



Fig. 5: Active cysts in the heart of a calf with clear cyst fluid and scolex, infected with 10 000 eggs (4 months post-infection).



Fig. 6: Diaphragm of a calf infected with 5000 eggs of Taenia saginata (30 days post infection).

and granulation-tissue formation.

The calves infected with 10 000 eggs of the parasite showed more or less similar tissue reaction but the intensity was a little greater at different stages of infection. At 2 months post-infection, the tissue reaction in heart was slight, with few inflammatory cells. However, the reaction in skeletal muscles was intense and numerous lymphocytes, plasma cells, eosinophils and macrophages infiltrated the lesions. The tissue reaction at 4 months post-infection was similar but more chronic in nature, with higher numbers of eosinophils and macrophages.

The calves that received 50 000 eggs of *T. saginata* revealed severe damage to the tissue and intense tissue reaction at 1 and 2 months post-infection. At 4 months post-infection, many cysticerci were degenerate (Fig. 10) and calcified in different body parts. There were zones of fibroblastic and granulation tissue around the cysts. Tissue reaction included necrotic areas and infiltration of lymphocytes, eosinophils, plasma cells and macrophages.

#### DISCUSSION

In the present experiment, all the calves aged between 1 and 2 months were highly susceptible to oral infection with 5000, 10 000 and 50 000 eggs of T. saginata. The calves showed clinical signs that included deviation in pulse and respiratory rates and a slight increase in body temperature in the 3rd week of infection. At a dose of 50 000 eggs, 1 calf developed tachycardia and others were also seriously affected. These findings regarding susceptibility of cattle to this infection conform with those of Froyd<sup>5</sup> who also observed high susceptibility in calves under 4 weeks of age. However, our observations on the clinical signs following this infection do not corroborate the initial statement of Blazek and Schramlova<sup>2</sup> that bovine cysticercosis is generally asymptomatic. We observed clinical signs in all calves infected with 5000, 10 000 and 50 000 eggs of the parasite. Blazek and Schramlova<sup>2</sup>, however, later stated that the clinical symptoms in this infection may appear at an early stage of heavy infection. Dewhirst et al.4 observed a decrease in haemoglobin content and PCV in cattle artificially infected with T. saginata and stated that these parameters could be used for antemortal diagnosis of this condition. They, however, observed little or no change in other blood parameters. Our results are more or less similar and there was regular decrease in Hb, RBC count and PCV in all the calves infected with



Fig. 7: Cyst in the forelimb muscles 2 months post-infection; wall (w), cavity (c), scolex (arrow) and sucker (s) and inflammatory cell reaction initiated by cysticerci (HE,  $\times$ 63).



Fig. 8: Microscopic changes in forelimb muscles due to cysticercosis at 2 months post-infection. Lymphocytes, plasma cells, macrophages and eosinophils infiltrated and the cyst is surrounded by connective tissue (HE,  $\times 250$ ).



Fig. 9: Body reaction to 4 months old cysts evidenced by infiltration of macrophages, eosinophils and lymphocytes. (HE,  $\times1000).$ 

5000, 10 000 and 50 000 eggs. In addition, we also observed an increase in WBC count and number of lymphocytes and eosinophils in the 3rd and 4th week of the infection. The pattern of haematological deviation could be an indication for clinical diagnosis subject to confirmation by other methods.

No uniform pattern of distribution of cysticerci in different body parts of experimentally infected calves was observed. However, the most seriously affected parts were masseter muscles and heart, followed by diaphragm and intercostal muscles. Tongue, muscles of fore- and hindlimbs and oesophagus showed moderate infection. The cysticerci were randomly distributed throughout the skeletal musculature. The results agreed with the observations of McCool<sup>10</sup> who also did not observe any uniform distribution pattern of cysticerci in the body of individually infected cattle. The present pathological study revealed insignificant gross lesions in most of the organs except skeletal muscles and diaphragm. The microscopic lesions in most of the affected organs consisted of cellular infiltration with lymphocytes, eosinophils, plasma cells and macrophages, necrosis of the tissue, proliferation of fibroblasts and development of granulation tissue along with the degeneration of cysts. These observations are in accordance with those of Silverman and Hulland<sup>12</sup>, who observed an acute to chronic inflammatory response and degeneration of cysts. More or less similar lesions in cattle have also been reported previously<sup>1,2,7,9,15</sup>. However, the lesions caused by different doses of infection at different intervals during this experiment showed a definite pattern, particularly in the development of cysts and tissue reactions.

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Fig. 10: Scolex (S) and the contents of a degenerate cyst in forelimb muscles 4 months post-infection (HE,  $\times 63).$ 

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