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PREVALENCE OF *DICROCOELIUM DENDRITICUM* INFECTION IN SHEEP AT TAIF PROVINCE, WEST SAUDI ARABIA

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Abstract

Dicrocoelium dendriticum is a common zoonotic parasite of sheep in many Regions of Saudi Arabia. In chronic infections, this parasite causes biliary cirrhosis in livers of cattle, sheep, or goats and leads to economic losses. This study compared the prevalence of *Dicrocoelium dendriticum* in local sheep and imported sheep in Taif Province, West Saudi Arabia. This cross-sectional survey was carried out in slaughterhouses in Taif Province. A total 2230 local sheep and 21383 imported sheep were studied. The number of injury in imported sheep was observed as 99 representing 0.46% of the number of animals examined, while no record of any injury was observed in local sheep.

Key words: Saudi Arabia, *Dicrocoelium dendriticum*, local sheep, imported sheep, liver infection.

Introduction

D. dendriticum or the lancet liver fluke is a parasite of the bile ducts and gall bladder of worldwide distribution among more than sixty mammalian hosts (Mas-Coma and Bargues, 1997). However, sheep, cattle and other ruminants are the true definitive hosts and the other definitive hosts including man are more or less mere alternative hosts. The human clinical symptoms of true infection are neither uniform nor specific (Wilson, 1991). *D. dendriticum* with *D. hospes* as being long and narrow, are generally confined to the more distal parts of the bile ducts. As a result most *D. dendriticum* infections of the biliary tree produce only mild symptoms. In the initial stages, there is leukocytosis, eosinophilia and traces of bile acids in the urine. On further infection, slight anaemia may ensue, the leukocytosis drops to normal level and eosinophila diminishes to 5% -7% (Rosicky and Groschaft, 1982). Usually, the dicrocoeliasis is accompanied by either a prolonged period of constipation or diarrhea, nausea and vomiting. Sometimes, patients may complain of abdominal discomfort and pain in the right half of the abdomen and in the epigastrium radiating to right shoulder and chronic watery diarrhea with slight elevation of liver function tests (Cengiz *et al*. 2010). Additionally, Manga-González *et al*. (2004) reported significant increases in hepatic enzyme activity (ALT & AST), which they suspected was a pathogenic response to parasite invasion which produces host-toxic metabolites. Sometimes, infection is accompanied by lassitude, headache and giddiness. Pain in the liver is continuous, independent of the uptake of food, and intensifies mainly at night (Price and Child, 1971).

Generally, parasitosis is regarded as an important health problem of grazing animals in Saudi Arabia, with losses associated with nematode and trematode infection approximating a million dollars (Mcleod, 1995). Reproductive disorders and parasitic infestation are the main problems that affecting productivity and cause great economic losses in farm animals (El-Khadrawy *et al*, 2008). Parasitic liver infections in meat producing animals are one of the major factors that reduce the national income and cause economic losses in this country (Radostits *et al*, 2007), either directly by their effect on animal growth and so meat production, or indirectly by affecting their reproduction (Senlik, *et al*, 2008). It has been found that parasitic helminthes are one of the most de-

structive internal parasites of vertebrate animals including man (Strickland, 2000).

Sheep received great interest as one of the most important livestock for human consumption in in all Arab Countries, especially the Gulf.

The aim of this study was to raise awareness of the zoonotic *Dicrocoelium dendriticum* and its pathogenicity by clarifying the histopathological changes by TEM.

Materials and Methods

The two slaughterhouse certified by Taif Municipality were explored for the survey. Samples were collected from daily visits in the period from November 2013 to January 2014 A total 2230 liver of local sheep and 21383 livers of imported sheep were examined (Ogambo-Ongoma, 1972) and identified (Soulsby, 1982). The total infection rate % in local sheep and imported sheep in Taif Province was calculated.

In the laboratory parasite were separated from infected tissue and examined using a Stereo light microscope as well as a Transmission Electron Microscope and histological sample were prepared (Tab. 1).

Table 1: technique of worm preparation for material s preparation for examination

Process	Material	Duration
1st Fixation	2.5% Glutaraldehyde (0.2 M phosphate buffer, pH 7.4)	2 h
Washing	Phosphate buffer wash	3 x 15 minutes each
2nd Fixation	1% OsO4 (buffered as above)	2 h
Washing	Phosphate buffer wash	3 x 15 minutes each
Dehydration	Ascending series of Ethanol	1 x 15 minutes each
Infiltration	Acetone	1 hr.
	Acetone : 1resin	2 x 1 hr. each
Embedding	Pure resin	1 hr
Blocks polymerization	Oven at 60^{o}C	3 days
Cutting	Ultrathin sectioning 60-90nm & gold on copper grids by ultra-microtome Leica model EM UC	
Contrasting	Tissues held on copper grids stained by Uranyl acetate and lead citrate (15 minutes r each).	
Examination	By Transmission Electron Microscope model Jeol JEM 1011	

Results

The results are shown in table (2) and figures (1 to 9)

Table 2: Statistics of local and imported infected sheep

Month	Total Number Imported	Total Number infection	Percentage of infection
November 2013	1482	5	0.33
December	1104	3	0.27
January 2014	1506	4	0.26
February	1413	7	0.49
March	1431	6	0.41
April	1392	3	0.21
May	1446	8	0.55
June	1927	10	0.51
July	1212	4	0.33
August	1430	6	0.41
September	1311	11	0.83
October	1621	21	1.29
November	1456	5	0.34
December	996	4	0.40
January 2015	1656	2	0.12

Discussion

In the present study, infection with *Dicrocoelium dendriticum* in sheep was all over the study period. The infection rate was changes between up and down There was marked increase in February followed by slight drop, then marked increase in May followed by slight drop. The infection rate increased again in September with a higher raise in October.

In the present study, the hyperplastic Kupffer phagocytic cells with numerous vacuoles and dense granules in cytoplasm (Figs. 3 & 4) also clearly visible with lower magnifications (X2500). Also, transmission electron micrographs (X10,000) showed hyperplasia in Kupffer cells with multiple cytoplasmic fat vacuoles scattered between cells (Fig. 5).

These caused indentations in the nuclei on the periphery against the cell membranes resulting in a signet ring appearance. Other histo-pathological abnormalities in infested hepatocytes revealed dense granular vesicular cytoplasm. Portal tract granuloma were seen constituting lymphoplasmocytes, histiocytes, foreign body giant cells and fibroblasts all surrounding the parasite (Fig. 6).

Moreover, the infected sheep livers demonstrated disarrayed hepatocytes with cytoplasm of different-sized black bodies (Fig. 7), the vacuolization and compressed blood sinusoids. The infected hepatocytes also showed cytoplasmic granules as well as scattered phagocytes and blood sinusoids in between cells containing proteinaceous material in addition to blood cells (Fig. 8).

Dicrocoelium dendriticum infection increased blood proteins, but Theodoridis *et al*. (1991) reported that the presence of up to 4000 worms in the host did not cause significant protein loss, thus not a serious threat. The extremely low numbers of *Dicrocoelium* reported in the present study had no serious effect on the sheep and apart from their pathological effect in the liver, had no effect on the economy.

In the present study, although infection was low in Taif Province and pathological symptoms were quite conspicuous, rendering them unsellable. Lesions and/or ulcers were the main reason for discarding all the infected sheep livers.

The most serious sign of *Dicrocoelium* infection was reduced weight in the early stages of infection, development of anemia, oedemas and reduction in production (Broglia *et al*, 2009).

Dicrocoeliasis was endemic or potentially endemic in 30 countries. *D. dendriticum* was found throughout Europe (former U.S.S.R., Switzerland, Italy, Germany, Spain, and Turkey), the Middle East (Iran, Saudi Arabia, Kuwait, Lebanon and Syria), Asia (China, Japan, and Vietnam), and Africa (Ghana, Nigeria, Sierra Leone, and Somalia) and in North and South America and Australia (Stancampiano *et al.* 2007). Human infection ranged between one patient in USA (Drabik *et al.* 1988) up to 208 patients in Saudi Arabia (el-Shiekh Mohamed and Mummery, 1990). WHO (2007) included *D. dendriticum* on its list of organisms to target with its Food-borne Disease Burden Epidemiology Reference Group.

In Arab Countries, infections in man and/or animals have been reported in Egypt (Scheid *et al,* 1950; Massoud *et al*, 2003; Haridy *et al*, 2003; 2006; El-Shafie *et al*, 2011), Iraq (Wajdi and Nassir, 1983), Lebanon and Syria (Yenikomshian and Berberian, 1934; Tohmé and Tohmé, 1977; Khalil *et al*, 2013), Kuwait (Al Behbehani *et al,* 2003), Somalia (Nødgaard and Kristensen, 1995), and in Sudan (Sabbatani and Fiorino, 2009).

On the other hand, in Saudi Arabia many authors reported the zoonotic dicrocoeliasis (Nasher, 1990; Omar *et al*. 1991; Gawish *et al*, 1993; Abu Zinada, 1999; Helmy and Al Mathal, 2003; Al-Mathal and Fouad, 2004; Al-Megrin, 2010; Mohammad and Koshak, 2011).

Conclusion

Dicrocoeliasis (Lancet liver fluke disease) is caused by *D. dendriticum*, a trematode living in bile ducts of sheep, cattle and other mammals including man. Human and animal infections have been world widely reported particularly in some of the Eastern Mediterranean sheep raising countries. The life cycle proceeds through two intermediate hosts: the land snail and the field ant. Zoonotic transmission could occur through the sheep, buffalo, deer and/or donkey. Human infection is acquired by consuming 17 species of field ant with raw fruits, vegetables, herbs or even with the drinking water. Human infection is asymptomatic or mild to moderately severe, but being sporadic or rarely reported. Basically, the public health workers and medical veterinarians must keep in mind zoonotic parasitosis such as the encountered and underestimated dicrocoeliasis.

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Explanation of figures

Fig. 1: Rate of injuries /month among study period.

Fig. 2: Ovine liver infected with *Dicrocoelium dendriticum*. Parenchyma hardened as a result of severe fibrosis and calcification (deformities in cellular and tissue structure), thickened ducts (A), and worms in tissue (B). Scale bar=5 cm.

Fig. 3: Parasite causing (A) multiple cytoplasmic electron dense globules (blue); (B) pushing and indenting nuclei (red); to periphery against cell membrane (E/M x8000).

Fig. 4: Parasitic infection indicated by (A) hyperplastic dense granule (upper left) (green) in cytoplasm (E/M x8000)

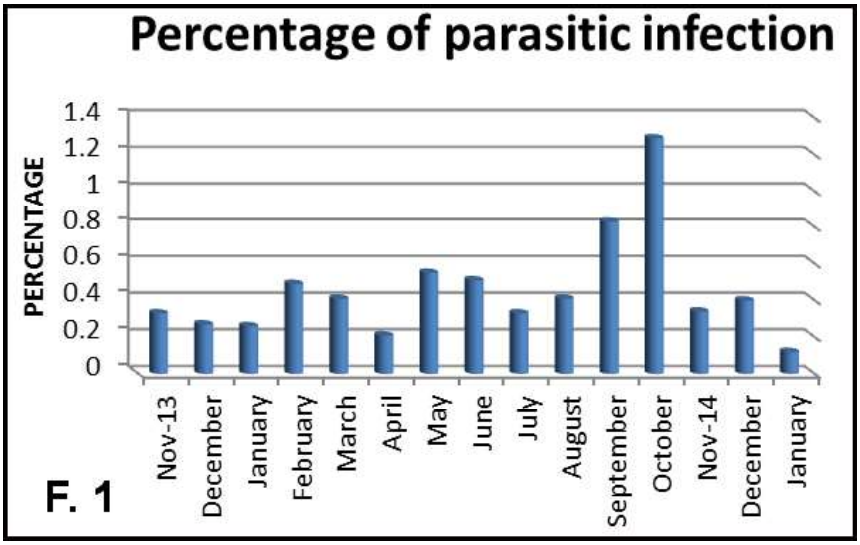
Fig. 5: Parasitic infection causing (A) Kupffer cell proliferation (red); and (B) multiple cytoplasmic electron dense granule (oranges) pushing nuclei to periphery against cell membrane (E/M, x2500).

Fig. 6: Parasitic infection causing (A) Kupffer cell hyperplasia (oranges); with (B) multiple cytoplasmic fat globules (blue); pushing (C) indent nuclei to periphery against cell membrane (red); giving signet ring appearance (E/M, x10,000).

F. 7: Parasitic infection with proliferated Kupffer cells and (A) hepatocytes possessing a granular vesicular cytoplasm(oranges); (B) portal tract granuloma (Red); formed by lymphoplasmocytes, histiocytes, foreign body giant cells and fibroblasts around parasite (Toluidine blue x 200).

Fig. 8: Infected disarrayed hepatocytes containing (A) cytoplasmic parasitic variable sized blackish bodies (red); and reveal (B) vacuolated granular cytoplasm. Blood sinusoids compressed (oranges). Toluidine blue x200.

Fig. 9: Infected (A) hepatocytes that contain cytoplasmic granules (yellow); and (B) centrally placed nuclei (oranges). Also, scattered phagocytic cells, blood sinusoids in between infected hepatocytes (C) compressed and contain blood cells and proteinaceous aterial (red); Toluidine blue, x200.


Percentage of parasitic infection
PERCENTAGE
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1.2
1
0.8
0.6
0.4
0.2
0
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February
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June
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August
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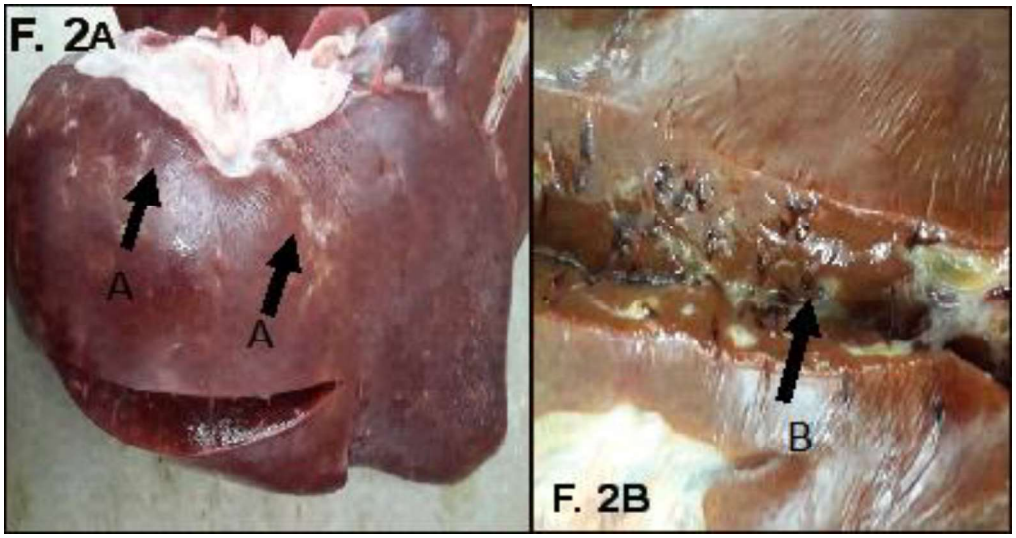
October
Nov-14
December
January
F. 1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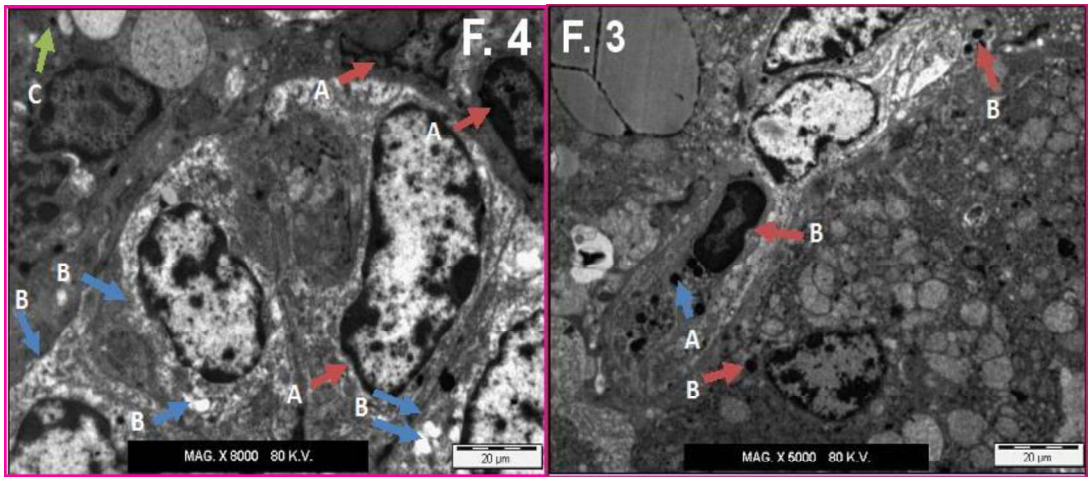
F. 2B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F. 3
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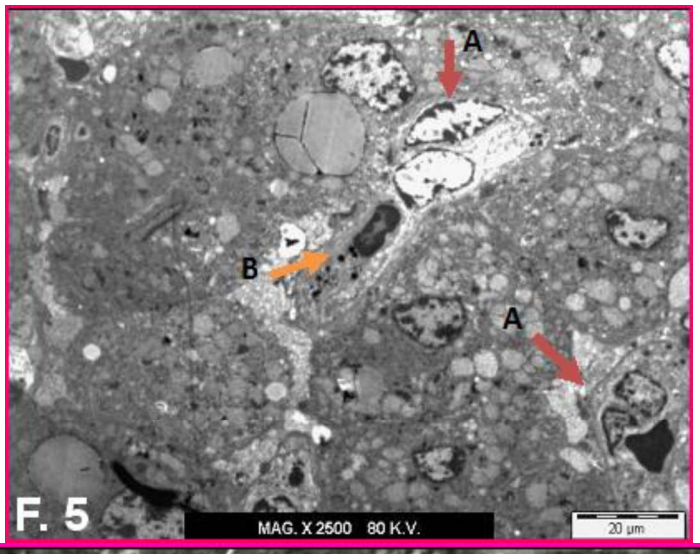
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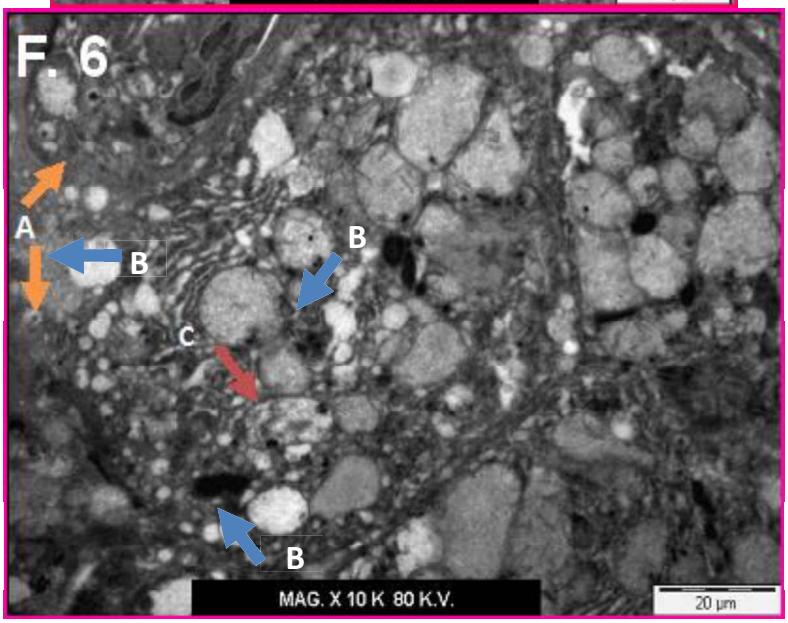
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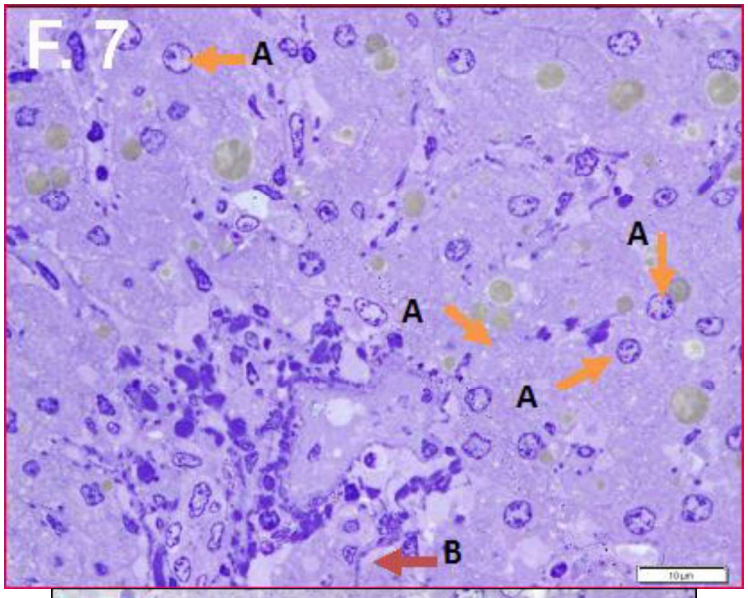
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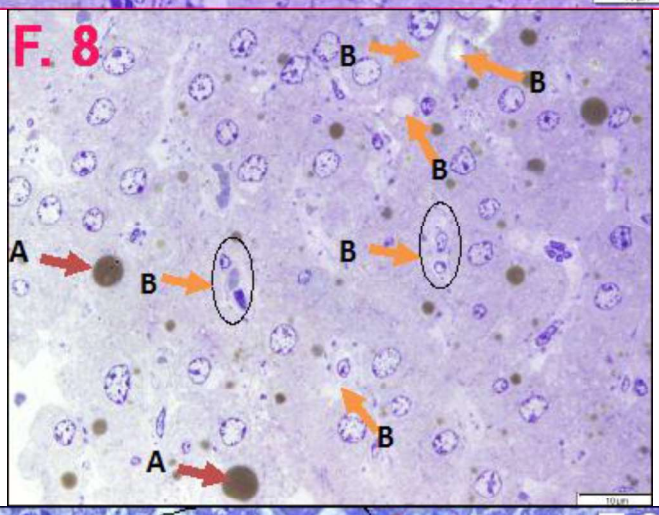
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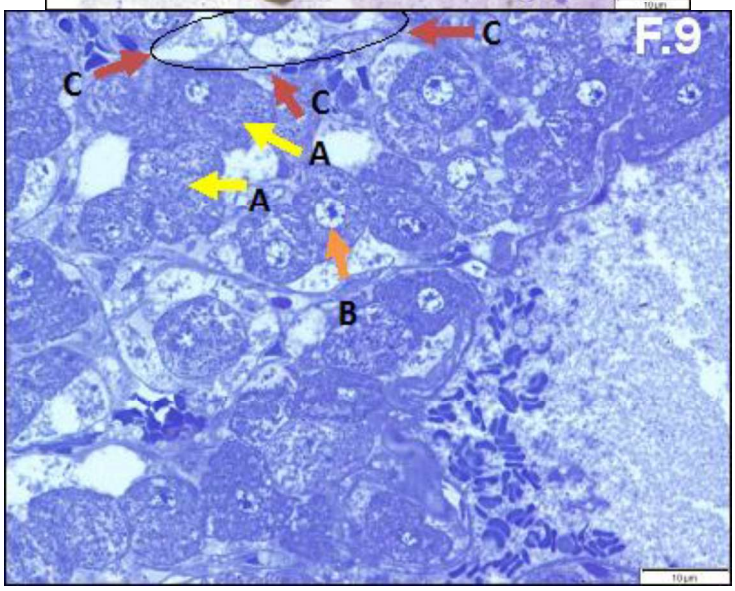
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