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# MORPHOMETRIC STUDY OF MICROSCOPIC CYSTS OF *SARCOCYSTIS* SP. IN SHEEP CARCASSES

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## ABSTRACT

**Muscular samples of the oesophagus and diaphragm of 335 sheep collected from the slaughterhouse of El Harrach were analyzed by the histopathological method to describe the morphology of two species of *Sarcocystis*: *S. arieticanis* and *S. tenella*. The cysts were counted and measured with a micrometer for their dimensions. A total of 895 cysts were measured. The width, length, shape index (length/width), the thickness of the wall and the length of the projections were recorded. The thick-walled cysts of *S. tenella* were 10—450 µm long (the mean ± SD value was 50.35 µm ± 1.380) and 1—110 µm (the mean ± SD value was 27.51 µm ± 0.533) wide. The cyst walls were 0.5—4 µm thick (the mean ± SD value was 1.547 µm ± 0.020) and provided with radial striations. The shape index was 1—14 µm (the mean ± SD value was 1.93 ± 0.045). While in the *S. arieticanis*, the cyst wall was thin and had long hair-like protrusions, the cysts measured 8—780 µm (the mean ± SD value was 123.13 µm ± 12.799) in length and 5—100 µm (the mean ± SD value was 37.00 µm ± 1.68) in width. The hair-like protrusions reached a length of 3—14 µm (the mean ± SD value was 5.428 ± 0.353) and the shape index was 1—17 µm (the mean ± SD value was 3.10 ± 0.281). For the length, the width and the shape index, the differences were statistically significant between thin-walled cysts and thick-walled cysts. The general structural features of the cysts, previously described by other authors, were confirmed.**

**Key words: histopathological method; *Sarcocystis arieticanis*; *S. tenella*; sheep**

## INTRODUCTION

*Sarcocystis* is a common coccidian parasite of a wide range of animals and is of worldwide distribution. It is commonly found in the cystic form in the musculature of slaughtered food animals [8].

Four species of *Sarcocystis* commonly infect sheep: *Sarcocystis ovifelis* (formerly *gigantea*) and *Sarcocystis medusiformis* are transmitted by felids and develop into macro-

scopically visible cysts in sheep, while *Sarcocystis ovicanis* (formerly *tenella*) and *Sarcocystis arieticanis* are transmitted by canids and develop into microscopic cysts in sheep [8]. Recently other species of *Sarcocystis* have been reported to occur in sheep including *Sarcocystis gracilis*-like [11] and *Sarcocystis mihoensis* [30].

Two *Sarcocystis* species are known to be pathogenic in sheep: *Sarcocystis tenella* and *Sarcocystis arieticanis* [8]. Upon primary exposure to pathogenic species, acute sarcocystiosis may cause death of the host, abortion, premature parturition in pregnant ewes [18], neurologic signs known as ovine protozoal myeloencephalitis (OPM) [3] and respiratory disease [31]; while chronic infections can lead to reduced quality of wool, meat and milk [21, 22].

The size, shape, and cyst wall morphology of sarcocysts are important in the identification and differentiation of *Sarcocystis* sp. [19, 24]. The species with the sheep-canid life cycle have far smaller cysts than the sheep-felid species [15, 25, 28, 29]. The cyst wall of *S. tenella* is observed thick by light microscopy but thin in the other 3 species, *S. arieticanis*, *S. gigantea* and *S. medusiformis*; *S. mihoensis* had the far thicker cyst wall than all the above 4 species [7, 8, 15, 19, 28, 29].

Many studies have shown that the sarcocyst wall varies structurally between species and it could be used as a useful criterion for the identification of species [2, 7, 12, 20, 25, 28]. There are numerous detailed investigations on the morphology of *Sarcocystis* sp. from domestic sheep [15, 26, 27, 28].

This paper describes the morphology of two microscopic species of sarcocysts; *S. arieticanis* and *S. tenella* in the muscles of two organs (oesophagus and diaphragm) of sheep slaughtered in the slaughterhouse of El Harrach in the north of Algeria. The present description is the first report from sheep in Algeria. There are different data on the size of the sarcocysts of both *Sarcocystis* species described here.

## MATERIALS AND METHODS

### Collection of tissue samples

Muscle samples were collected from 335 sheep carcasses slaughtered at a slaughterhouse of El Harrach in the north of Algeria. A total of 670 muscle samples from 335 oesophagi and 335 diaphragms were sampled and placed into separate bags. Samples were taken to the Laboratory of Parasitology and Mycology in the Superior National Veterinary School, Algiers and were previously fixed in 10 % neutral buffered formalin for histological analysis of microscopic *Sarcocystis* cysts.

### Histological technique

Tissue samples were prepared in the Laboratory of Anatomy and Cytopathology of the University Hospital of PARNET, Algeirs, and in the Laboratory of Anatomy Pathological at the Superior National Veterinary School, Algiers. The fixed tissue samples were cut into 0.5 cm-thick sections, dehydrated with serial dilutions of ethanol and xylene, processed into paraffin, sectioned of 4 to 5 microns, stained with haematoxylin and eosin (H & E), and examined for microcysts with the light microscope at various magnifications (×100, ×400, ×1000). The wall morphology of the microcysts in the muscle sections were examined by the light microscope with oil immersion (×1000). Sarcocystis species present in the muscle samples were identified on the basis of the differences in the wall morphologies of sarcocysts, as described by D u b e y et al. [11]. The cysts were counted and measured with a micrometer under a light microscope in order to determine their dimensions. The width, the length, the shape index (length/width), the thickness of the wall and the length of the projections were recorded.

### Statistical analysis

The data were processed using IBM SPSS Statistical version 20 software. Means, standard errors and 95 % confidence intervals were determined for the different morphological characteristics. For the comparison, the Mann-Whitney U-test and the Chi-square test were used for the study of homogeneity. A difference was considered statistically significant at $P < 0.05$.

## RESULTS

*Sarcocystis* sp. infections were diagnosed in 315 (94.03 %) of the sheep. Sarcocysts were located within the muscle cells. Two types of microcysts were differentiated on the basis of their cyst wall morphologies under the light microscope (×1000). Some cysts were bounded by a thin cyst wall, with the hair-like projections identified as *S. ar-*

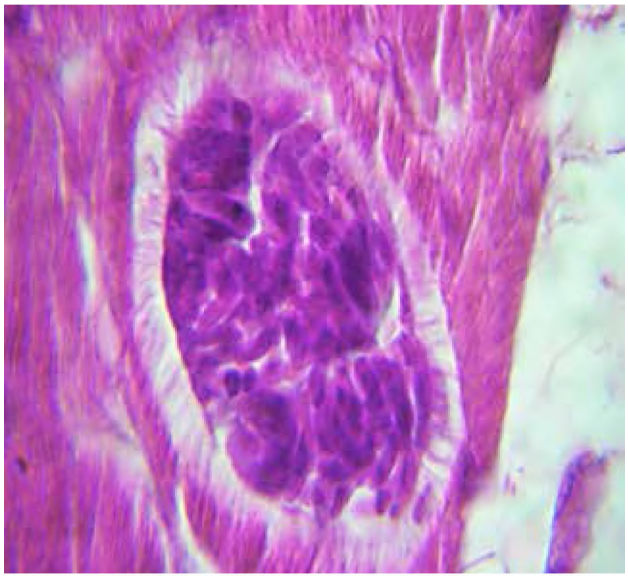

**Fig. 1. *S. arieticanis*, thin-walled with hair-like projections in oesophagus (H&E, Magn. ×1000)**

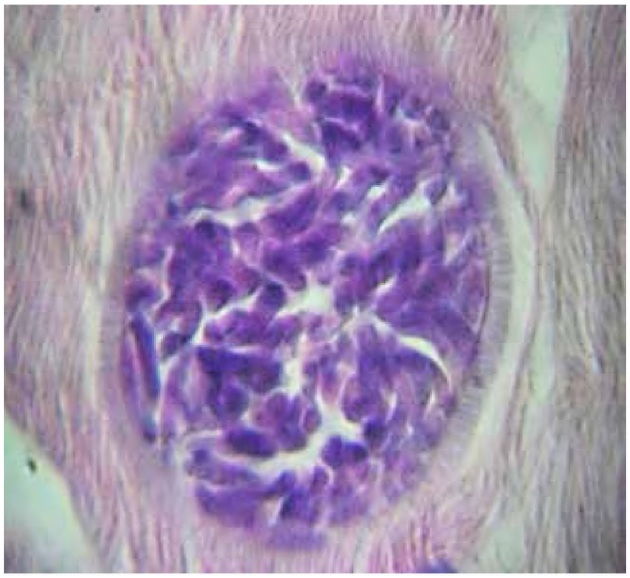

**Fig. 2. *S. tenella*, thick-walled with radial striations in oesophagus (H&E, Magn. ×1000)**

*ieticanis* (Fig. 1). Other cysts were bounded by a relatively thick cysts wall with radial striations which were identified as *S. tenella* (*S. ovicanis*) (Fig. 2). Among the cysts found, 895 cysts were measured (772 thick-walled cysts of *S. tenella* and 123 thin-walled cysts of *S. arieticanis*).

**Structural features of the cysts**

The means of length, width, shape index, thickness of the wall (*S. tenella*) as well as the length of the projections (*S. arieticanis*) are reported in Table 1. For the length, the width and the shape index, the U de Mann-Whitney test was very significant ($P < 0.01$) between thin-walled cysts and thick-walled cysts.

The means of length, width, shape index, thickness of the wall (*S. tenella*) and the length of the projections (*S. arieticanis*) according to the organ are found in Tables (2) and (3). For each *Sarcocystis* species, the U Mann-Whitney test showed no significant differences ($P > 0.05$) between the two organs for each parameter measured.

**Thickness of the wall (thick-walled cysts) and length of the projections (thin-walled cysts)**

The U Mann-Whitney test demonstrated that the distribution of the number of thin-walled cysts of *S. arieticanis* according to the length of the projections (villi) was not significant between the 3 classes, while for the thick-walled cysts, the distribution according to the thickness of the walls was highly significant (Table 4).

**Length of the cysts**

For the thick-walled cysts, the highest number of cysts was in the class ≤ 50 µm, followed by the class [51—100 µm], and ≥ 100 µm; a highly significant difference was found between the 3 classes. For the thin-walled cysts, the class ≤ 50 µm represented the highest number of cysts, followed by the class ≥ 100 µm, then the class [51—100 µm]; the test of U Mann-Whitney showed that there was not a difference between the 3 classes (Table 5).

**Width of the cysts**

Regarding the width of the cysts, a very large number of cysts belonged to the class ≤ 50 µm, followed by the class [51—100 µm] for the two species of *Sarcocystis*; for the class ≥ 100 µm, we founded only one cyst of *S. tenella*, while no cyst was found for *S. arieticanis*. The statistical analysis showed that there was a highly significant difference between the three classes for each *Sarcocystis* species (Table 6).

**Shape index of the cysts**

The sarcocysts in the oesophagus and diaphragm were round (Fig. 3), ovoid (Fig. 4), fusiform (Fig. 5), or linear (Fig. 6). The distribution of cysts according to the shape index showed that a very large number of cysts were found in

**Table 1. Morphological characteristics of thick-walled and thin-walled cysts**

| | Parameters | Length [µm] | Width [µm] | Shape index [µm] | Wall thickness of thick-walled cysts [µm] | Length of the projections of thin-walled cysts [µm] |
|---|---|---|---|---|---|---|
| **Thin-walled cysts** | n | 123 | 123 | 123 | – | 35 |
| | Mean ± SD | 123.13 ± 12.79 | 37.00 ± 1.68 | 3.10 ± 0.28 | – | 5.42 ± 0.35 |
| | 95 % CI | [97.79—148.47) | [33.68—40.33] | [2.54—3.66] | – | [4.71—6.14] |
| | Min | 8 | 5 | 1 | – | 3 |
| | Max | 780 | 100 | 17 | – | 14 |
| **Thick-walled cysts** | n | 772 | 772 | 772 | 772 | – |
| | Mean ± SD | 50.35 ± 1.38 | 27.51 ± 0.53 | 1.93 ± 0.04 | 1.547 ± 0.02 | – |
| | 95 % CI | [47.64—53.06] | [26.47— 28.56] | [1.84—2.01] | [1.50—1.58] | – |
| | Min | 10 | 1 | 1 | 0.5 | – |
| | Max | 450 | 110 | 14 | 4 | – |
| **P value** | | **0.000** | **0.000** | **0.01** | **–** | **–** |

CI—Confidence Interval

**Table 2. Morphological characteristics of thick-walled cysts according to the organ**

| Thick-walled cysts | Parameters | Length [µm] | Width [µm] | Shape index [µm] | Wall thickness [µm] |
|---|---|---|---|---|---|
| **Oesophagus (n = 330)** | Mean ± SD | 47.61 ± 1.65 | 27.82 ± 0.81 | 1.84 ± 0.063 | 1.54 ± 0.032 |
| | 95 % CI | [44.35—50.87] | [26.22—29.42] | [1.71—1.96] | [1.483—1.610] |
| | Min | 10 | 1 | 1 | 1 |
| | Max | 300 | 89 | 13 | 4 |
| **Diaphragm (n = 442)** | Mean ± SD | 52.40 ± 2.064 | 27.28 ± 0.707 | 1.99 ± 0.062 | 1.548 ± 0.026 |
| | 95 % CI | [48.34—56.46] | [25.89—28.67] | [1.87—2.11] | [1.49—1.59] |
| | Min | 10 | 4 | 1 | 0.5 |
| | Max | 450 | 110 | 14 | 4 |
| **P value** | | **0.932** | **0.579** | **0.496** | **0.656** |

CI—Confidence Intervals

**Table 3. Morphological characteristics of thin-walled cysts depending on the organ**

| Thin-walled cysts | Parameters | Length [µm] | Width [µm] | Shape index [µm] | Villous length [µm] |
|---|---|---|---|---|---|
| **Oesophagus (n = 42)** | Mean ± SD | 132.79 ± 21.35 | 39.64 ± 3.18 | 3.20 ± 0.46 | 5.27 ± 0.51 |
| | 95 % CI | [89.66—175.91] | [33.22—46.07] | [2.26—4.13] | [4.17—6.36] |
| | Min | 8 | 5 | 1 | 3 |
| | Max | 500 | 100 | 13 | 10 |
| **Diaphragm (n = 81)** | Mean ± SD | 118.12 ± 16.04 | 35.64 ± 1.94 | 3.05 ± 0.35 | 5.55 ± 0.49 |
| | 95 % CI | [86.20—150.04] | [31.77—39.51] | [2.35—3.76] | [4.51—6.59] |
| | Min | 8 | 7 | 1 | 4 |
| | Max | 780 | 80 | 17 | 14 |
| **P value** | | **0.402** | **0.247** | **0.763** | **0.705** |

CI—Confidence Interval

**Table 4. Distribution of the number of thin-walled cysts according to the length of the projection and of thick-walled cysts according to the thickness of the wall**

| Villous length [μm] | N | P | Wall thickness [μm] | N | P |
|---|---|---|---|---|---|
| [3—4] | 12 | | [0.5—1] | 311 | |
| [5—6] | 17 | 0.074 | [1.5—2] | 397 | 0.00001 |
| [7—14] | 6 | | [2.5—4] | 64 | |
| **Total** | **35** | **Not significant** | **Total** | **772** | **Very significant** |

**Table 5. Distribution of the number of thin-walled cysts and thick-walled cysts according to the lengths of cysts**

| Length of thick-walled cysts [μm] | N | P | Length of thin-walled cysts [μm] | N | P |
|---|---|---|---|---|---|
| ≤ 50 | 499 | | ≤ 50 | 52 | |
| [51—100] | 226 | 0.0001 | [51—100] | 30 | 0.052 |
| ≥ 101 | 47 | | ≥ 101 | 41 | |
| **Total** | **772** | **Very significant** | **Total** | **123** | **Not significant** |

**Table 6. Distribution of the number of thin-walled cysts and thick-walled cysts according to the width of the cysts**

| Width of thick-walled cysts [μm] | N | P | Width of thin-walled cysts [μm] | N | P |
|---|---|---|---|---|---|
| ≤ 50 | 718 | | ≤ 50 | 103 | |
| [51—100] | 53 | 0.00001 | [51—100] | 20 | 0.00001 |
| ≥ 101 | 1 | | ≥ 101 | 0 | |
| **Total** | **772** | **Very significant** | **Total** | **123** | **Very significant** |

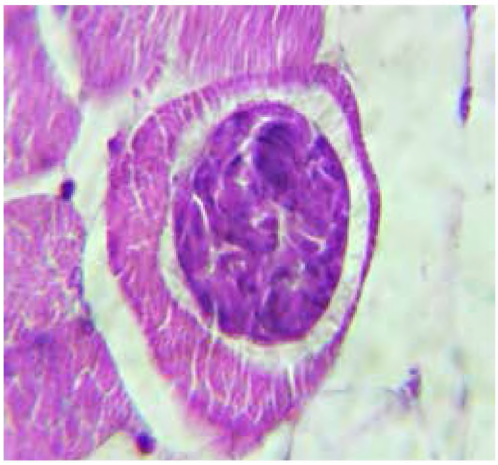

Fig. 3. *S. arieticanis*, round, thin-walled with hair-like projections in diaphragm (H&E, Magn. ×1000)

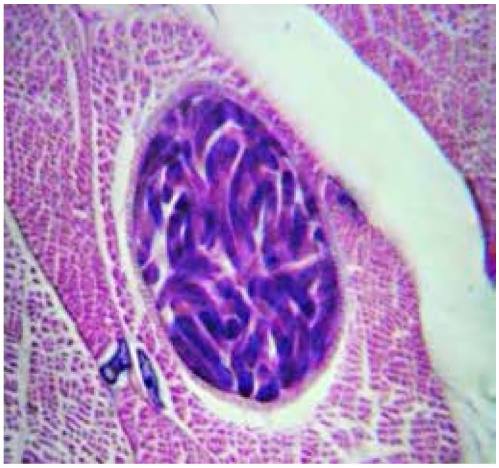

Fig. 4. *S. tenella*, ovoid, thick-walled with radial striations in oesophagus (H&E, Magn. ×1000)

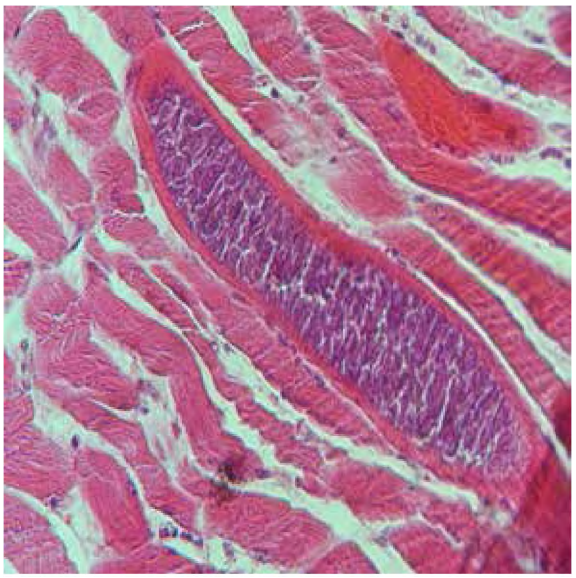

Fig. 5. *S. arieticanis*, fusiform, in diaphragm (H&E, Magn. ×400)

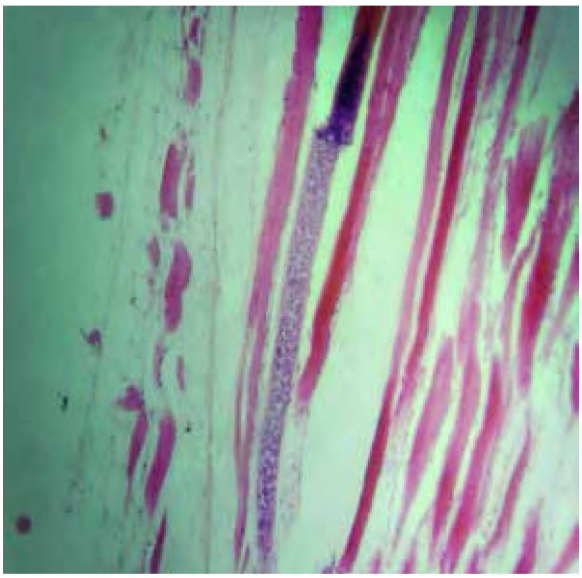

Fig. 6. *S. arieticanis*, linear, in diaphragm (H&E, Magn. ×100)

**Table 7. Distribution of the number of thin-walled cysts and thick-walled cysts according to the index of forms cysts**

| Index of forms of thick-walled cysts [µm] | N | P | Index of forms of thin-walled cysts [µm] | N | P |
|---|---|---|---|---|---|
| ≤ 1.19 | 169 | | ≤ 1.19 | 30 | |
| [1.2—2] | 389 | 0.00001 | [1.2—2] | 41 | 0.052 |
| ≥ 2.01 | 214 | | ≥ 2.01 | 52 | |
| **Total** | **772** | **Very significant** | **Total** | **123** | **Not significant** |

the class [1.2—2 µm] for *S. tenella* and the class ≥ 2.01 µm for *S. arieticanis*; the statistical tests showed that there was a highly significant difference between classes for the thick-walled cysts, while this difference was not significant for the thin-walled cysts (Table 7).

## DISCUSSION

*Sarcocystis* species parasitize almost all vertebrates and are widely distributed around the globe. Data from various studies suggest different variations depending on the species and the area where the research was performed; for sheep the prevalence is between 18 % and 100 % [4]. In Algeria, the prevalence of microscopic cysts of *Sarcocystis* has been reported to be between 64.38 % and 99.14 % [5, 23]. Two types of microscopic cysts were detected with differences in their cyst walls morphologically: *S. tenella* and *S. arieticanis*. Sheep become infected with *S. tenella* or *S. arieticanis* by ingesting sporocysts in contaminated food or water [8].

In our study, the morphometric studies of the microscopic cysts of *Sarcocystis* (length, width, shape of cysts [length/width], and wall thickness of cysts) from slaughtered naturally infected sheep were carried out.

In the 335 sheep examined, cysts were detected in the oesophagus and the diaphragm in great numbers. The *sarcocystis* were located within the muscle fibers and there was no evidence of any pathological changes or reaction against the cysts. The interior of the mature cyst was divided into

many chamber-like compartments by septa. A great number of bradyzoites were observed in the cyst together with a small number of metrocytes which were situated along the cyst wall.

There is remarkable diversity in cyst wall zone morphology between different *Sarcocystis* species and this variation has been used as a means of species identification [13, 19].

In our study, the cyst wall of *S. tenella* was thick and striated; the same as that of *S. tenella* described by Heydron et al. [14] and Vlemmas et al. (35). No secondary cyst wall was observed in our study which was in agreement with Vlemmas et al. [35]. While in *S. arieticanis*, the cyst wall was thin and had long hair-like protrusions, which was similar to that described previously for this parasite [12, 15, 26].

In Algeria, there is very little data on the occurrence of the species of ovine *Sarcocystis* spp. Indeed, Dahmani et al. [5] recorded that 92.54 % of the sheep examined were infested with the thin-walled cysts of *S. arieticanis*, and 43.88 % of sheep with the thick-walled cysts of *S. tenella*. Nedjari [23] noted the predominance of *S. tenella* (60.63 %) compared with *S. arieticanis* (39.36 %).

There has been different data reported on the size of the sarcocysts of both *Sarcocystis* species described here. Some authors (Vercruysse et al. [34], Kudi et al. [17]) have identified the *Sarcocystis* species based on the dimensions of the cysts. Thus, the measurements of the different dimensions (length, diameter, thickness) of the cysts obtained, in our study, are compatible with the species of *S. tenella* and *S. arieticanis*.

Indeed, the cysts of *S. arieticanis* measured 8—780 μm (the mean ± SD value was 123.13 μm ± 12.799) in lengh and 5—100 μm (the mean ± SD value was 37.00 μm ± 1.68) in width. Tenter [32] reported that cysts of *Sarcocystis arieticanis* are 900 μm or less in length. Our results are almost similar to those found by some authors. In fact Al Quraishy et al. [2] found in the heart muscles that the cysts measured 38.5—64.4 μm (averaged 42.66 μm) in width and 62.4—173.6 μm (averaged 82.14 μm) in length, also within cardiac muscle of naturally infected sheep. Haziroglu et al. [12] recorded that the cysts were ranging over 35.0—62.5 μm in width and 52.5—162.5 μm in length. On the other hand, in a study conducted by Odening et al. [26] on fresh samples of muscle tissue (head, neck, larynx, heart and loin: *Musculus psoas major*) from European mouflon and domestic sheep, the sarcocysts were up to 1.8 mm long and 286 μm wide.

In our study, it was demonstrated that the cyst wall of *S. arieticanis* cysts was readily identifiable by light microscopy, having a cyst wall < 1 μm characterized by hair-like protrusions. These protrusions reached a length of 3—14 μm (the mean ± SD value was 5.428—0.353). The same results were noted by Odening et al. [26] who recorded that the cyst walls were thin (0.21 to 0.62 μm) and they had unstable hair-like, 5.7 to 11.8 μm long villar protrusions. Also, Heydorn and Mehlhorn [15] found that the tissue cysts of *S. arieticanis* were characterized by hair-like protrusions reaching a length of 11 μm at the maximum. Dubey et al. [7] noted that *S. arieticanis* showed a thin wall with filiform projections with 6—12 μm.

We found *S. tenella* in the sheep we examined. The sarcocysts were 10—450 μm long (the mean ± SD value was 50.35 μm ± 1.380) and 1—110 μm (the mean ± SD value was 27.51 μm ± 0.533) wide. According to Tenter [32] the cysts *S. tenella* were 700 μm or less in length. Our results were close to those recorded by Tinak [33] who found that *S. tenella* cysts measured 68.591 μm in length and 23.260 μm in diameter. Kudi et al. [17] noted that the sarcocysts of *S. tenella* measured 35.7 to 500 μm lengthwise in tissue samples of the oesophagus and diaphragm. While according to Vercruysse and Van Marck [34], the cysts have an average length of 300 μm. Abd el-Baki et al. [1] found in the oesophagus samples that the mature sarcocysts measured 700 (670 —860) μm in length and 188 (170—200) μm in width in the longitudinal sections, while it was measured 75 (22—118) μm in the cross sections. Whereas, Erber [9] showed that *S. tenella* measured 300—650 × 20—50 μm. For Odening et al. [26] the sarcocysts were up to 2.4 mm long and 186 μm wide which was different from our results.

Fassi-Fehri et al. [10] found, that regarding the length of the cysts, the highest number of thick-walled cysts of *S. tenella* (139) was in class [100—300 μm], followed by 47 cysts in class [0—100 μm], and 29 cysts in class [300—500 μm], while 5 cysts had a length which was > 500 μm.

We recorded that the cyst walls were 0.5—4 μm thick (the mean ± SD value was 1.547 μm ± 0.020). Similar results have been noted by Odening et al. [26] who found that the cyst walls were 1.08 to 3.85 μm thick and Tinak [33] who recorded 1.081 μm thick. From Kudi et al. [17]

the cyst-wall measured 2.4 µm. Whereas K a n a k o u d i s et al. [16] recorded that the cyst wall appeared uniformly thick with numerous digit-like projections and were 3—3.5 µm thick.

From F a s s i - F e h r i et al. [10], regarding the wall thickness of the *S. tenella* cysts, a very large number of cysts (195) belonged to the class [1—4 µm], followed by the class > 5 with 30 cysts.

This difference may be related to the methods of measuring. Indeed, certain authors have resorted to the electron microscopy for this measurement [34]. This is why other techniques (molecular biology, immunohistochemistry) must be used for the more precise identification of *Sarcocystis* species [6].

The shape index (length/width) was calculated to indicate the shape of the cyst; indeed for thin-walled cysts of *S. arieticanis*, the shape index was 1—17 µm (the mean ± SD value was 3.10—0.281) while it was 1—14 µm (the mean ± SD value was 1.93—0.045) for thick-walled cysts of *S. tenella*. The sarcocysts in the oesophagus and diaphragm were round, ovoid, fusiform or linear. It is suggested that their shape varies with the arrangement of the myofibrils where they are located and they are dependent upon the section. T i n a k [33] noted that the *Sarcocystis* cysts observed were of variable shapes (elongated or ovoid) depending on the section. In longitudinal sections, the cysts were fusiform, linear, while in cross-section they were round. F a s s i - F e h r i et al. [10] found that most cysts were fusiform or linear and out of 201 *Sarcocystis tenella* cysts measured, 25 cysts had a shape index > 2, whereas for 176 cysts, this index was between 2 to 16.

## CONCLUSIONS

Our investigation descibed the morphology of two microscopic species of *Sarcocystis*; *S. arieticanis* and *S. tenella* from the oesophagus and diaphragm of slaughtered naturally infected sheep for the first time in Algeria. The width, the length, the shape index (length/width), the thickness of the wall of *S. tenella* and the length of the projections of *S. arieticanis* recorded by other authors were confirmed.

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