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IDENTIFICATION OF*SARCOCYSTIS CRUZI, SARCOCYSTIS HIRSUTA* AND *SARCOCYSTIS HOMINIS* IN SLAUGHTERED COWS IN KAZERUN THROUGH PATHOLOGY AND DIGESTION PROCEDURES

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ABSTRACT

One of the usual domestic ruminator protozoans is sarcocystis parasite. *Sarcocystiscruzi*, *Sarcocystishirsuta* and *Sarcocystishominis* are three species of Sarcocystis that caused cyst in cow muscles and infect human. All over the world and in Iran, Sarcocyst is spreading highly in cow muscles, but there is no sufficient study about Sarcocyst species in Iran.The aim of the study is identification of slaughtered cows according to morphology features in Kazeroon city. Through this study,51 diaphragms of cows are collected from Kazeroon slaughter house. Then through digestion and pathology procedures, the samle regarding bradyzoite and sarcocyst cyst for recognition of *Sarcocystiscruzi*,*Sarcocystishirsuta* and *Sarcocystishominis* types, survey via optical microscope. Through digestion procedure and via optical microscope, it is specified that all of the sample infected by sarcocystbradyzoite , through pathology procedure via optical microscope observed two kinds of cysts with thin and thick septum. Regarding acquired results by the two procedures, *Sarcocystiscruzi* contained bradyzoites with length of 6-

18/68micrometers and with average of 9/71 micrometers and width of 2/33-5/98 micrometers and average of 3/88 micrometers and contained cysts with diameter less than 1 micrometer. *Sarcocystishirsuta* contained bradyzoites with length of 16/08-28/51 micrometers and average of 18/51 micrometers and width of 6/12-2/54 micrometers and average of 4/15 micrometers and contained diameter of 2/03-5/44 micrometers. *Sarcocystishominis* contained bradyzoites with length of 6/27-15/23 micrometers and average of 9/28 micromters and width of 2/44-5/76 micrometers and average of 3/77 micrometers, contained cysts with diameter of 1/11-5/49 micrometers. Therefore regarding acquired results of two procedures, the three species of *Sarcocystiscruzi*, *Sarcocystishirsuta* and *Sarcocystishominis* are confirmed and recognized.

INTRODUCTION

Sarcocystis is one of the widespread parasites in domestic pack animals. Firstly, the parasites whit white fleck and length of 1-3 centimeters at mouse muscles reported by Misher without a scientific name. Sarcocyst have compulsive life cycle with two hosts, at this cycle, the parasite contains two reproduction processes sexual and asexual. Asexual process contains shizogony and cyst formation in the middle host and sexual process contains gametogony, fertility and sporogony in the last host. The last host infect with eating beef and viscera that contains adult sarcocysts of middle host, and finally excretes infective sporosyst along with its excrement[1,2].

Sarcocystis species have long and thin shapes or crescent that named bradyzoites[3]. Bradyzoites are in the sarcocystis cyst and can observe with microscope. Heydoran andhis co-workers (1975) for the first time collected observations that there are three species of sarcocystis in cow with sexual processes in human, dog and cat, and there are two species of sarcocysts in sheep with sexual processes in dog and cat[4]. So far, several species of parasites in human and animals are recognized, some of the important parasites in cow are *Sarcocystis bovicanis* or *S.cruzi*, *S.bovifelies* or *S.hirsuta*, *S. bovihominis* or *S.hominis*. The certain host of this species are sequancelycanis, catties and primates especially human[5,6]. *S. cruzi* creates microscopic cysts and usually hare the length of less than 500 micrometers, the last host are dog and other canis , the most pathogen species of parasite is in cow and has global dispersion[3,6]. *S. hirsuta* creates macroscopic cysts, cat is the last host, creates light pathogenic[7] and often has no signal clinical. *S.hominis*[1] creates microscopic cysts in cow muscles, the last host is human.

Consuming beef whether raw or underdone the *Sarcocystshominis* infecthuman, and caused dyspepsia like as nauseam, stomachache and diarrhea [8]. there are few ways for recognition of sarcoystis species that morphological features of sarcocysts enjoy serious circumastance at appointing species[3,9,10] in cow there are different studies about sarcocysts features and some factors as length of cyst, width, septum diameter, shape and fuzzy outstanding size of cyst septum,the angel among fuzzy outstanding and the level of cyst septum and inner structures of fuzzy outstanding are recognized through optical and electronic microscope [3,11,12,13] .The rate of infected quarrel by the parasites is estimated about 70-100 around different world area. Some of the sarcocystis species are important pathology organisms that are dangerous for human and quarrelsand caused sickness that call sarcocystosis .The harmful effects of the pathogen parasite mainly occurred in middles hosts but the infecting in last host is light[3,14].

Sarcocystosis is light in live animals according to normal qualifications and have unperceivableclinical signs, but sometimes causes death in infected animals[3, 15]. The cow is one of the middle hosts of sarcocystis species, the rate of spreading sarcocyst in adult cow muscles is very high and is near 100 percent in the most world areas that studied[16]. The rate of infecting carcasses of slaughtered cows by sarcocystis is very high in Iran, conversely there is not enough studies about recognition of sarcocystis species in cow. The aim of the studies was recognition of sarcocystis species in slaughtered cows in Kazeroon through pathology and digestion procedures to recognize the importance of general hygienic.

MATERIALS AND METHODS

In Kazerun slaughter house 51 cows are evaluated. Sampling accomplished from diaphragm muscle. The sample tested diagnostically by two digestion and pathology procedures.

Pathology procedure

From each sample a diameter of 1 centimeter is cutted and fix in formalin discretely. After abiding samples, preparing tissues and production of sliced paraffin formats colored as hematoxylin-eosin. And then survey through optical microscope withmagnification of ×100 regarding sarcocystis cyst. So, for exact determination of sarcocystis species the diameter of observed cysts septum measured with optical microsecope according to micrometer, and study septum shape.

Digestion procedure

At this procedure 20 grams of diaphragm muscle squashed with meat grinder and then mixed with 50 milliliters digested dilution that contain 1/3 gram of pepsin powder, 2/5 grams of salt and 3/5milliliters hydrocholoridric acid in 500 milliliters aquapura . Then put the sample container in the waterbath of 40 degrees for 1 hour and 30 minutes after liquidizing samples, centrifuge liquidizing dilution for 5 minutes with revolution of 2500. Then throw away the liquid over it and from alluvia and survey through optical microscope with magnification of 100 regarding sarcocyst bradyzoit. Then for exact appointment ofsarcocystis species, the length and width of observed bradyzoits measure through optical microscope per micrometer.

RESULT

From 51 cows studying with optical microscope through producing histopathology sections, 37 of them infected to sarcocyst cyst. The cyst observed in two kind: thin and thick. The diameter of observed septum cysts, measured by optical microscope per micrometer and also study the shape of septum. From 51 cows studying with optical microscope through digestion procedure All of the cows infected to sarcocystbradizoite. The length and width of observed bradyzoite measured with optical microscope per micrometer. Therefore regarding this two kinds, three species of sarcocystis are recognized.

1. *Sarcocystbovicanis* or *Sarcocystiscruzi* species: the species that have cyst with thin septum. The diameter of cysts septum (1-1 figure) was less than 1µm (table 1).At the outer level of septum of cysts observed salientsciliaryvillar. The species had bradyzoites (2-1 figure) with length of 6-18/68µm (average=9/71µm, s=2, n=326) (table 2) and width of 2/33-5/98 (average=3/88µm, s=0/71, n=326) (table 3).

   The baradyzoites and cysts of this species observed in 21 cows.

2. *Sarcocystisbovifelise* species or *Sacocystishirsuta*: they had cysts with thick septum. The diameter of septums was 2/03-5/44 µm (table 4). At outer level of cysts(figure3), observed marbled salient through optical microscope.

   The salient observed like tressed hedge and glossa shape. Meanwhile, this salient contained different flection. This species had bradyzoite with length of 16/08-28/51 µm

(figure 4) (average=18/51µm, s=2/54, n=25) (table 5) and width of 2/54-6/12 (average=4/15 µm, s=/80,n=25) (table 6) bradyzoite and cysts of this species observed in 2 cows.

3. *Sarcocystisbovihominis* species or *Sarcocystishominis*: The species that have cysts with thick septum. The diameter of septum was 1/11-5/49µm (table 7). The thickseptum of cysts observing whether smooth (figure 5) or marbled(radial) salient (figure 6) at the outer level of cyst septum through optical microscope. The salient was hedge and fixed in cyst level like a stature design. The salient toward sarcocystishirsuta is recognizable lessly and for bearing of flections status, the salient toward sarcocystis hirsuta sarcocystis was packed mostly .This species have bradyzoites (7 figure) with length of 6/27-15/23µm(average=9/28, s=2/06, n=127)(table8) and width of 2/44-5/76µm(average=3/77µm, s=/72, n=127) (table 9) bradyzoites and cysts of this species observed in 14 cows.

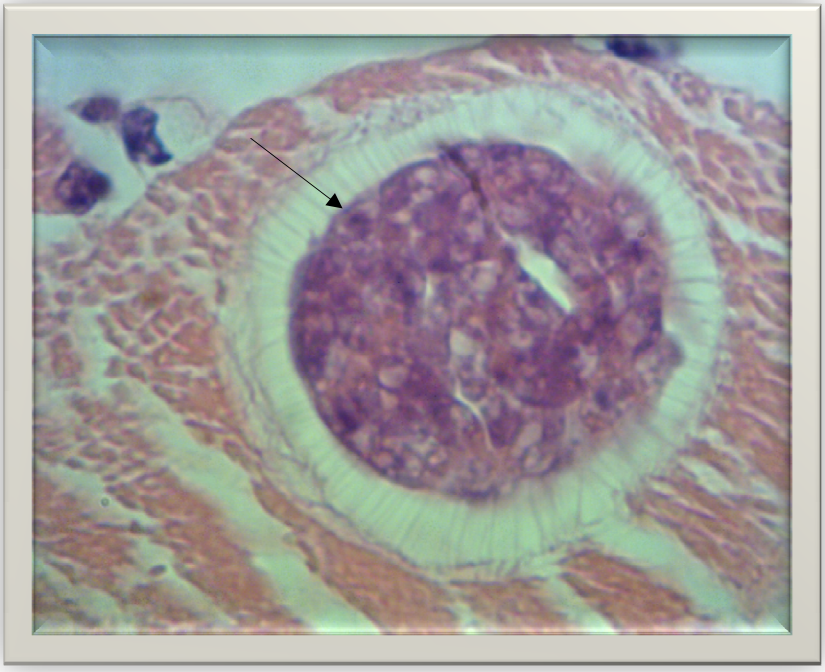

Figure 1: optical photomicrograph is from thin septum of *Sarcocystisbovicanis* cyst that their ciliarysalients are shown with Fletcher. The optical photomicrograph is with magnification of ×7500 and colored with hematoxylin –eosin.

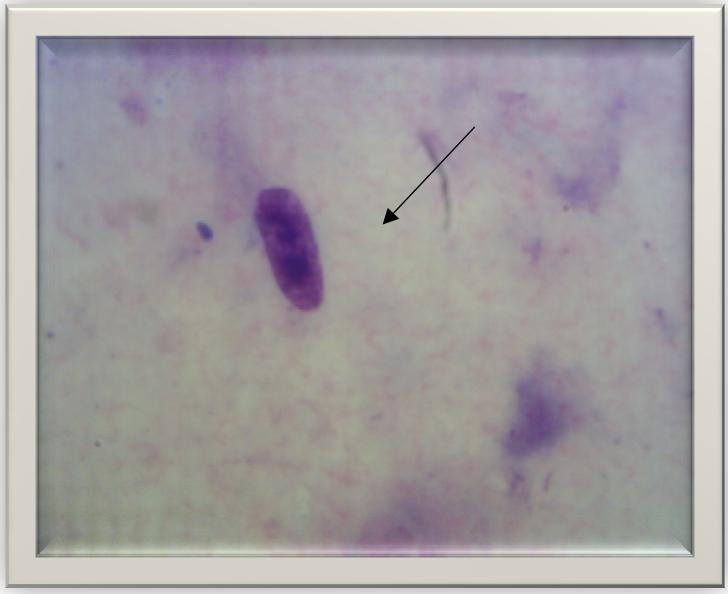

Figure2: Optical photomicrograph is from bradyzoite of *Sarcocystisbovicanis* species that is shown with a Fletcher. The optical photomicrograph is with magnification of ×7500 and colored with giemsa.

Table 1: The diameter of cyst septum of sarcocystiscruzi species per micrometer through pathology procedure

Animal species	Number of infective sample	Diameter of cyst septum (per micrometer)	Cyst nature	Sarcocystisspecies
Cow	21	Less than 1	microscopic	Sarcocystiscruzi

Table 2: Range, average and standard deviation of length of bradyzoites of *Sarcocystiscruzi* per micrometer through digestion procedure

Animal species	Number of slides	Length of Range	average	Standard deviation
Cow	326	6-18/68	9/71	2

Table 3: Range, average and standard deviation of width of bradyzoites of *Sarcocystiscruzi* per micrometer through digestion procedure

Animal species	Number of slides	Width of range	Average	Standard deviation
Cow	326	2/33-5/98	3/88	/71

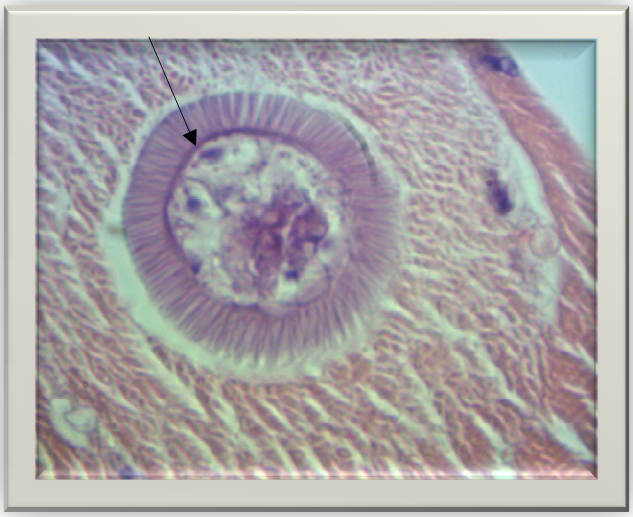

Figure 3: optical photomicrograph is from thick septum of *Sarcocystisbovifelise* cyst that its marbled salient is shown with Fletcher. The optical photomicrograph is with magnification of ×7500 and colored with hematoxylin-eosine.

Figure 4: optical photomicrograph is from bradyzoite of *Sarcocystisbovifelise* species that observed with Fletcher. The optical photomicrograph is with magnification of ×7500 and giemsa coloring.

Table 4: The diameter of cyst septum of *Sarcocystishirsuta* species per micrometer through pathology procedure

Animal species	Number of infective kinds	Diameter of cyst septum (per micrometer)	Cyst nature	Sarcocystisspecies
Cow	2	2/03-5/44	microscopic	*Sarcocystishirsuta*

Table 5: Range, average, standard deviation of length of bradyzoite of *Sarcocystishirsuta* per micrometer through digestion procedure

Animal species	Number of slide	Length of range	average	Standard deviation
cow	25	16/08-28/51	18/51	2/54

Table 6: Range, average and standard deviation of width of bradyzoites of *Sarcocystishirsuta* per micrometer through digestion procedure

Animal species	Number of slide	Width of range	Average	Standard deviation
Cow	25	2/54-6/12	4/15	/80

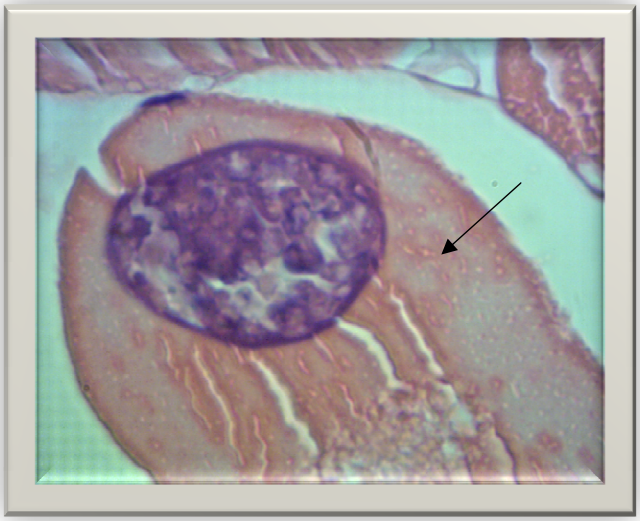

Figure 5: optical photomicrograph is from thick and smooth septum of *Sarcocystisbovihominis* that is shown with Fletcher. The optical photomicrograph is with magnification of ×7500 and hematoxylin-eosin coloring.

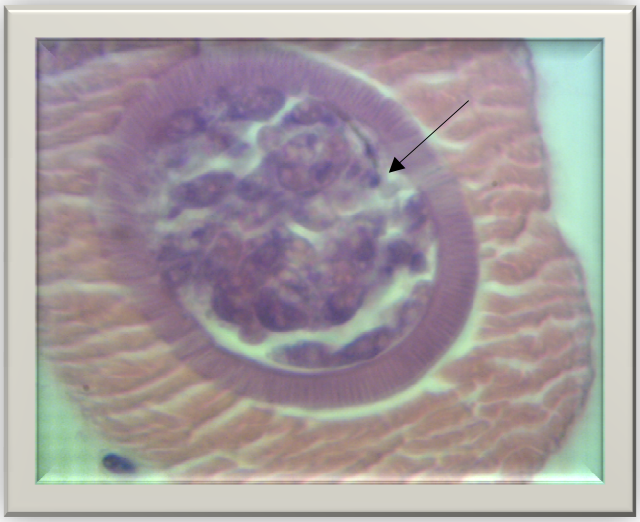

Figure 6: optical photomicrograph is from thick septum cyst of *Sarcocystisbovihominis* that is shown marbled , radial and stature salient of them with feltcher . The optical photomicrograph with magnification of X7500 and hematoxylin-eosin coloring.

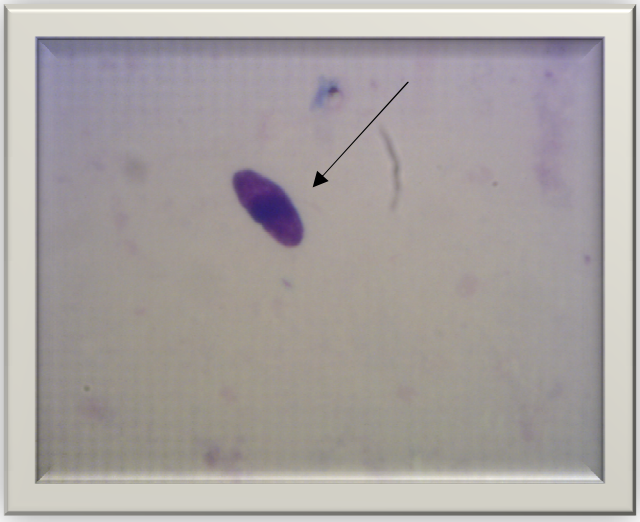

Figure7: The optical photomicrograph is from *Sarcocystisbovihominis* species that is shown with feltcher. The optical photomicrograph is with magnification of X7500 and giemsacoloring .

Table 7: The diameter of cyst septum of *Sarcocystishominis* species per micrometer through pathology procedure

Animal species	Number of infective kinds	Diameter of cyst septum(per micro meter)	Cyst nature	Sarcocystisspecies
cow	14	1/11-5/49	microscopic	*Sarcocytishominis*

Table 8: range, average and standard deviation of length of bradyzoites of *Sarcocystishominis* per micrometer through digestion procedure

Animal species	Number of slides	Length of range	average	Standard deviation
Cow	127	6/27-15/23	9/28	2/06

Table 9: range, average and standard deviation of width of bradyzoites of *Sarcocystishominis* per micrometer through digestion procedure

Animal species	Number of slides	Width of range	average	Standard deviation
Cow	127	2/44-5/76	3/77	/72

DISCUSSION AND RESULT

The present research aiming recognition of *Sarcocystiscruzi*, *Sarcocystishirsuta* and *Sarcocystishominis* in the muscles of slaughterd cows in Kazerun city accomplished byoptical microscope.Diagnostic technique was according to shape and measuring of cyst septum and bradyzoites. from all of the surveyed samples can conclude that 100% of samples was infected protozoan sarcocyst and observed 3 species of cow sarcocystis in the samples. the accomplished researches were about recognition of named species , majority and done by microscopic research under optical microscopy , ultra research by electronic microscope and molecular surveys. Badawy and his coworkers (2012) at the sarcocystishominisservey and other in factious cow sarcocystis at sharkia province Egypt observed cysts with thick and thin septum under optical microscope . They named cysts with thin septum ,*Sarcocystiscruzi* is and are reported less than 1 micrometer the diameter of cyst septum with thin septum[17] . That the diameter of obtained *Sarcocystiscruzi* cyst at this research matches with the result of *Sarcocystiscruzi*cyst diameter at the present research.HosseinNourani and his coworkers (2010) at surveging spreading of *Sarcocystiscruzi* with thin septum and *sarcocystishirsuta* or *Sarcocystishominis* with thick septum from Iran cows , with optical microscope and through histopathology procedure can observe cysts with thin septum and this cysts accommodate with thin septum of *Sarcocystiscruzi*[18]. Shekarforoush and his coworker (2013) at the first recognition of the*Sarcocystishirsuta* of cow in Iran , recognize cysts with septum diameter of 1/56- 1/78 micrometers that had ciliarysalients shape . Studying about sub – structure of named cysts than was seeable with macroscope , reported *Sarcocystishirsuta* species[19]. Fatma and his coworkers (2008) at surveging the infection of sarcocystis in the slaughtered cows at Assiut slaughterhouse through microscope and serologic, and according to cyst size , morphology cyst septum and creating experimental infectious in dog , reported microscopic cyst of *Sarcocystiscruzi*[20] . Morihirosaito and their coworkers(1999) at the first detachment of *Sarcocystishominis* of slaughtered cows in Saitama after doing segmental steps , the tissue segments infected to cyst are surveyed by optical and electronic microscope . At last, the cysts sizes under the optical microscope were 1/220- 4/460x80-384 micrometers.The diameter of cyst septum under the optical microscope was 3-6

micrometers. Meanwhile, the cysts septum under optical microscope had villar hedge salient that observed as marbled. But under electronic microscope, the size of salient were 3.1-4/ 3×7-1/17 micrometers .meanwhile, this salient under electronic microscope had several microtubule in the center. Therefore, Marihirosaito and his coworkers recognized *Sarcocystishominis* under electronic and optical microscope[4]. Ifeomanancy,obijiaku and coworkers(2013)at surveying infectious to sarcocyst in slaughtered cows in Zaria of Nigeria slaughterhouse regarding presence sarcocysts and bradyzoite through digestion and pathology procedure under optical microscope, reported the cysts with thin septum and the diameter less than 1 micrometer and *Sarcocystishominis* with thick septum 3/61-7/22 micrometers[21] that the obtained cyst septum diameter of *Sarcocystiscruzi*cyst at this research is accommodated with cyst septum diameter of *Sarcocystcruzi* at present research. Odening and coworkers (1994) at surveying bison bonasus and bovidae as middle host , Three species of sarcocystis (from Apicomplexa and sarcocystidae family) in cow, express that *Sarcocystiscruzi* under optical microscope had nearly 1 micrometer diameter and had ciliary salient that the salient were not integrated or were stick in cyst septum but cyst septum of *Sarcocystishirsuta* contained tressed hedge salient. The salient were glossa shape and had different flections.The septum of *Sarcocystishominis* cyst was hedge toward *Sarcocystishirsuta* were recognizable lessly, forbearing of flections status, *Sarcocystishominis*salient toward sarcocystishirsuta were packed mostly[13]. The obtained results from cyst septum of three species at this research is accommodate with obtained results at the present research , also Odening and coworkers (1994) reported the bradyzoites of *Sarcocystiscruzi* with length of 15/8-17/6µm (average=17/3µm, s=1/12,n=30) and width of 2/6-3/2µm (average=2/95µm, s=/14,n=30), bradyzoites of *Sarcocystishirsuta* with length of 16/2-19/1µm (average=17/6µm,s=1/30, n=30) and width 2/6-2/9µm (average=2/8µm,s=/12,n=30)and bradyzoites of *Sarcocystishominis* with length of 12/9-14/7µm (average=13/9µm,s=/66,n=30) and width of 2/4-2/9 µm(average=2/8µm,s=/19,n=30) (13) that obtained results of bradyzoites accommodate nearly with present research.Moreover of named studies through optical microscope that are done byresearch regarding cow sarcocyst and studies accomplished by

electronic microscope and molecular technique among the surveys can point to Dubey and coworker(1989) the difference ultra-structure between sarcocystishirsuta and hominissarcocystis[9]. analysis of gen 18 srDNA of sarcocystishominis , sarcocystiscuzi, sarcocystishirsuta species by PCR technique aiming recognition of named species done by most researchers[22, 23,24,25]. The present research surveying cyst features and bradyzoite at the same time can recognize with comparing the two named cow sarcocystis species very carefully and subtract with each other. Therefore regarding the result of this research, the presence of three species of *Sarcocystishominis*, *Sarcocystishirsuta* ,*Sarcocystiscuzi*, admitted and confirmed in Kazeroun slaughterhouse through morphologicalfeatures and under optical microscope in slaughtered cows.

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