Revue Agrobiologia
www.agrobiologia.net
ISSN (Print): 2170-1652
e-ISSN (Online): 2507-7627

QUALITATIVE DATA AND DISTRIBUTION OF THE OOCYSTS EXCRETION IN THE RABBIT *ORYCTOLAGUS CUNICULUS* (LINNE, 1758) IN KABYLIA (ALGERIA)

NEBRI Rachid[1*], MEDROUH Bachir[1] et DOUMANDJI Salah Eddine[2]

1. Institut des Sciences Vétérinaires, Université de Blida1 (UN0901), BP270, Route de Soumaa, Blida, Algérie
2. Laboratoire de Recherches en Protection des végétaux ENSA (ES1603), Rue Hassen Badi Belfort El Harrach, Alger, Algérie

Reçu le 05/10/2022, Révisé le 13/05/2023, Accepté le 05/06/2023

Summary

Description of the subject: Plenty of worldwide studies were made on the identification and the causal agents biology of liver and digestive rabbits coccidiosis. In Algeria, cuniculture has experienced a big increase in the past few years especially in rural areas; as a result of this expansion coccidiosis is one of the constraints to its development as it remains one of the most important infectious causes of the digestive disorders fattening rabbits
Objectives: In order to identify coccidia of the genus Eimeria, agents of rabbit coccidiosis (Oryctolagus cuniculus (Linné, 1758), a survey was conducted on rabbit farms established in Kabylia (Algeria).
Methods: The study was carried out in three intensive farms chosen according to the relief and the climate. The first station is Ouacifs located in the mountains; the second Makouda is located in the middle mountains and the third, that of Tigzirt, is located on the Mediterranean coast. Several loose stool samples were taken.
Results: The faeces were treated using a coprological test and the analysis revealed the presence of seven species: *Eimeria stiedae* (Leeuwenhoek, 1674), *E. magna* (Pérard, 192), *E. media* (Kessel, 1929), *E. coecicola* (Cheissin, 1947), *E. vejdovskyi* (Pakandl, 1988), *E. flavescens* (Marotel and Guilhon, 1941) and *E. irresidua* (Kessel and Jankiewicz, 1931). Statistical analysis was carried out to establish the distribution of these species between the three stations. The results show the presence of four Eimeria assemblages linked to the location of the rearing sites and whose greater availability is in favor of a grouping equidistant from the three stations used to experimentation.
Conclusion: This work has made it possible to identify a new species for Djurdjura (Kabylia), namely *E. flavescens* which appears to be highly pathogenic. *E. flavescens* could rage in Kabyle rabbit farms and therefore harm livestock in the region.
Key words: Altitude, Coccidiosis, Distribution, Kabylie, Rabbit, Stools

DONNÉES QUALITATIVES ET RÉPARTITION DE L'EXCRÉTION DES OOCYSTES CHEZ LE LAPIN *ORYCTOLAGUS CUNICULUS* (LINNE, 1758) EN KABYLIE (ALGÉRIE)

Résumé

Description du sujet : De nombreuses études mondiales ont été réalisées sur l'identification et la biologie des agents causals de la coccidiose hépatique et digestive du lapin. En Algérie, la cuniculture a connu une forte augmentation ces dernières années, notamment en milieu rural ; Du fait de cette expansion, la coccidiose est l'une des contraintes à son développement car elle reste l'une des causes infectieuses les plus importantes de troubles digestifs chez le lapin.
Objectifs : Afin de répertorier les coccidies du genre *Eimeria*, agents de la coccidiose du lapin (*Oryctolagus cuniculus* (Linné, 1758), une enquête a été menée sur les cunicultures implantées en Kabylie (Algérie).
Méthodes : L'étude a été menée dans trois élevages intensifs choisis en fonction du relief et du climat. La première station est celle des Ouacifs située dans les montagnes ; le deuxième Makouda est située dans la moyenne montagne et la troisième, celle de Tigzirt, est située sur la côte méditerranéenne. Plusieurs échantillons de selles molles ont été prélevés.
Résultats : Les matières fécales ont été traitées à l'aide d'un test coprologique et l'analyse a révélé la présence de sept espèces : *Eimeria stiedae* (Leeuwenhoek, 1674), *E. magna* (Pérard, 192), *E. media* (Kessel, 1929), *E. coecicola* (Cheissin, 1947), *E. vejdovskyi* (Pakandl,1988), *E. flavescens* (Marotel et Guilhon, 1941) et *E. irresidua* (Kessel et Jankiewicz, 1931). Une analyse statistique a été pratiquée pour établir la répartition de ces espèces entre les trois stations. Les résultats montrent la présence de quatre assemblages d'*Eimeria* liés à la localisation des lieux d'élevage et dont la plus forte disponibilité est en faveur d'un groupement équidistant des trois stations ayant servis à l'experimentation.
Conclusion : Ce travail a permis d'identifier une nouvelle espèce pour le Djurdjura (Kabylie), à savoir *E. flavescens* qui apparaît comme hautement pathogène. *E. flavescens* pourrait sévir dans les élevages de lapins kabyles et par conséquent nuire au cheptel de la région.
Mots clés:. Altitude, Coccidiose, Distribution, Kabylie, Lapin, Selles

*Auteur correspondant : NEBRI Rachid, E-mail : nebri.rachid@gmail.com

INTRODUCTION

Plenty of worldwide studies were made on the identification and the causal agents biology of liver and digestive rabbits coccidiosis. In Algeria, cuniculture has experienced a big increase in the past few years especially in rural areas; as a result of this expansion coccidiosis is one of the constraints to its development as it remains one of the most important infectious causes of the digestive disorders fattening rabbits. The work that has been done in relation to the etiology of this parasitic disease remains very limited; however, it is worth mentioning the work of Nebri & Benkacemi [1] and of Henneb & Aissi [2] that addressed the identification of rabbit coccidia parasites in farms located in Mitidja and Kabylia, respectively. Coccidiosis is caused by an intracellular protozoan parasite of *Eimeria* genus, capable of generating a significant mortality rate in domestic rabbits. On the other hand, the majority of known species cause morbidity due to weight loss and diarrhea; Pakandl [3, 4], states that remarkable disturbances are observed in the production and during gestation of *Oryctolagus cuniculus*. The large proliferation of these protozoa and the absence of maternal-fetal immunity transmission make coccidiosis the major cause of digestive parasitic diseases in rabbit livestock. The same authors maintain that an *E. intestinalis* ingested oocyst produces $1.^{10}$ to $3.^{10}$ new oocyst. Many writers including El-Shahawi and *al*. [5] confirm that 15 species of rabbit-infecting coccidian *Eimeria* were identified. Of the ten *Eimeria* species (Schneider, 1875), coexisting among wild rabbits in France nine of which were identified from the oocysts morphology

MATERIALS AND METHODS

1. Study area and period

During the years 2020-2021 soft stools were collected from three rational breeding locations in the coast and mountains of Kabylia. sporulated oocysts were sought out for identification by the flotation technique.

.

-Ouacifs station : This station is 1638 meters high in Djurdjura mountain chain (36°31’ 59.9880’’ N ; 4° 13’ 0.0120 " E.). It is equipped with 126 cages, 88 for the maternity unit and 38 are used for fattening. The two batches are separately lodged in the same building.

-Makouda station : The farm is located in highland at 910 meters high (36°47’31.3706’’ N.; 4°3’44.6944’’ E). It contains two compartments, one with 100 cages for maternity and the other, also with 100 cages, is for fattening.

-Tigzirt Station : This station is on the sea coast (36°53’28.4748’’N ; 4°7’23.5812’’ E). It has a total of 140 cages portioned between two buildings. The first with 60 cages is allocated to maternity and the second with 80 cages is for fattening. This farm is influenced by the neighboring Mediterranean sea that belongs to the sub humid climatic zone with a hot winter.

2. Material

To take the stool samples, latex gloves were used. Faeces were put in plastic airtight bags and sent in an icebox to the laboratory.

-Samples : For each of the maternity and fattening sets, the livestock is divided up into ten batches numbered from 1 to 10. Two selections of stool samples were made randomly between the ten batches after drawing lots. Samples were taken regularly every week in the morning by placing collecting nets with extremely small links, the day before collection. After that, the stool samples put in airtight bags are transported to the laboratory for stool analysis. A total collection of 384 soft stool samples, spread over one year, was executed in 2020-2021.

-Laboratory equipment : The equipment utilized for faeces analysis in the laboratory consists of tools used in stool processing, observation instruments and additives.

-Stool treatment equipment: The stool processing equipment includes a bucket, mixer, pestle, mortar, beaker, precision balance, a tea strainer, funnel, test tube racks and bowls.

-Analysis equipment: It comprises slides, covers, an optical microscope, a digital camera, and immersion oil. Additives: $MgSO_4$, NaCl, bleach, NaOH, and distilled water.

3. Methods

The stool examination was performed on samples left at room temperature for a period of 4 to 7 days. The adopted technique is flotation. Based on a simple and practical procedure, it is a method of excreta treatment for coccidia identification.

-Operating method: The stool-containing bag under goes a brewing that leads to a vigorous harmonization. Then an aliquot sample of 300 g is removed to which a few drops of NaOH or failing that, of bleach are added. Next, an amount equal to 5 times this weight of distilled water is added and a second homogenization is required with soaking for one hour. After a thorough agitation, 40 g are collected, sieved and then rinsed twice with 30 g of $MgSO_4$ or NaCl. It is convenient to carry out the adjustment of the obtained filtrate amount to 100 ml with $MgSO_4$ or NaCl; tubes of the suspension are filled delicately to avoid the formation of air bubbles that may be annoying when observing. The filling is done so as to obtain a convex meniscus for each tube and then a cover is placed on each of these tubes. After 48 hours they are collected then laid on micro slides for microscopic observation. The diagnosis of the various perceived coccidia oocysts is carried out based on the descriptions reported by Eckert and *al*. [6], .and Coudert and *al*. [7].

-Statistical analysis: The leading aim of this study is first to count the coccidia infesting each considered rabbit farm, then to learn their diversity and distribution according to the farm location, and finally to specify the existing affinity between the identified species. To better illustrate these aspects, a correspondence analysis or C.A is performed. This multivariate mathematical analysis, results in a representation of 2- or 3-dimensions space of a set of points. This statistical review was conceived using the software Past ver. 3.0. The coding below was designed to allow the software recognize the data.

1: Coccidia Presence in the site; 0: Absence of coccidia in the site; **E. irr*:*** *E. irresidua;* **E. coe***: E. coecicola;* **E.psti***: E. stiedae;* **E. mag***:E. magna;* **E. roo***: E. roobroucki;***E. Pir***: E.piriformis;* **E. int***: E. intestinalis;* **E. med***: E. media;* **E. exi***: E. exigua;* **E. Per***: E.perforans;* **E. fla***: E. flavescens;* **E. vej***: E. vejdovskyi.*

RESULTS

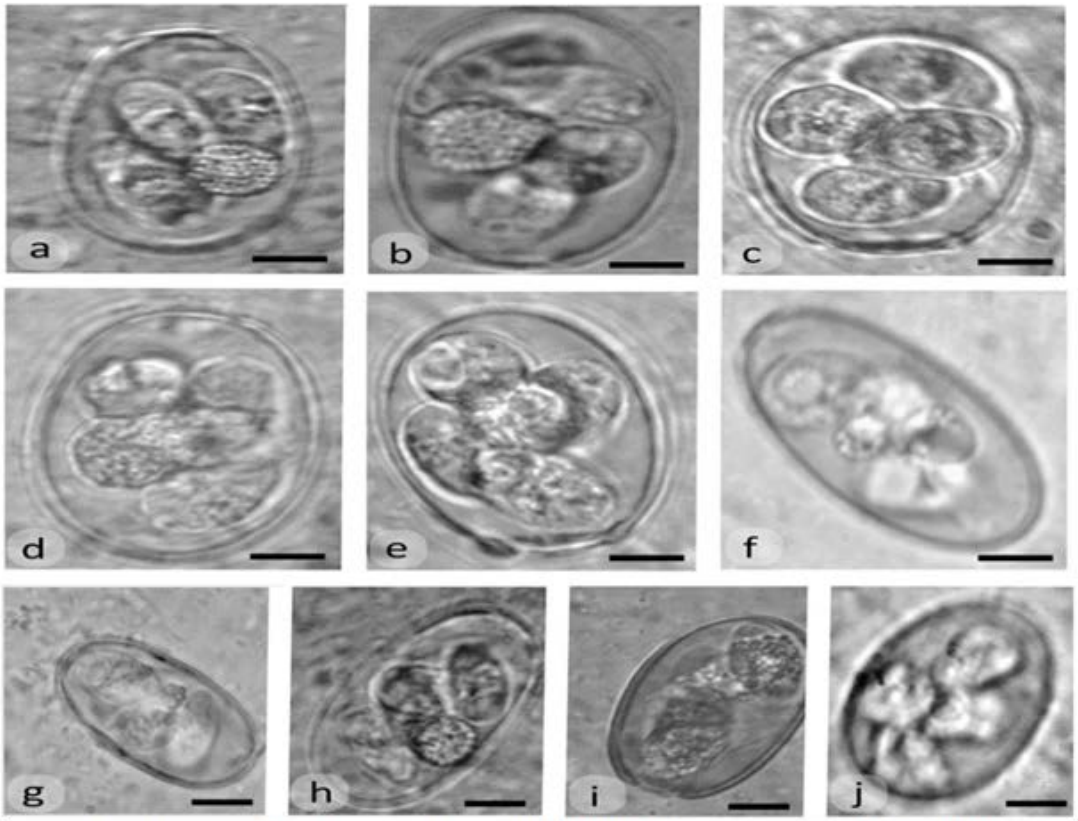
1. Species identification in the three breeding locations

This study reveals the existence of oocysts belonging to seven *Eimeria* species within examined rabbit's tools in three farms of Kabylia (Fig. 1) (Tab. 1). As to the measurements, the ellipsoid shape of the sporulated oocysts, the presence of a medium residual oocystic body and of a large micropyle which were discernable on each of the oocysts suggest that the latter are *Eimeria media* (Fig. 1a and b); (Tab. 1). The (Fig. 1c) shows a sub spherical sporulated oocyst with absence of residual body and a wide micropyle, indicating that this would be an *Eimeria flavescens* oocyst. Regarding (Fig. 1) (Tab. 1), sporulated oocysts of ellipsoid shape are perceived with a large oocystic residual body and a micropyle marked on both sores which indicate that they are *Eimeria magna* oocysts. In (Fig. 1f) (Tab. 1), it is plain to see a sporulated oocyst of ellipsoid shape with a small residual body and a flat micropyle; this oocysts undoubtedly an *Eimeria vedjovskyi*. (Fig. 1g and h) (Tab. 1) presents sporulated oocysts of elongated ovoid shape with a relatively small residual body and a narrow micropyle apparent on each of these protozoa which maintain that they are *Eimeria coecicola* oocyst. The sporulated oocyst with a wide and flat micropyle (Fig. 1i), (Tab. 1), given its size and the micropyle's shape, it corresponds to an *Eimeria irresidua oo*cyst. The sporulated oocyst, in the absence of micropyle and residual body, is that of *Eimeria stiedae* (Fig. 1i); (Tab. 1).

2. Results of the statistical test

The presence or absence of described Eimeria species in literature related to the infection of the rabbit *Oryctolagus cuniculus* in the study stations are mentioned in table 2. **S**pecies having affinities not only between themselves but also with their collecting sites are mentioned in small circles (Fig. 2). The correspondence analysis compared to the distribution of *Eimeria* oocysts sampled at the three locations is adequate as long as the sum of the two axes exceeds on the basis of a similarity of -1, 2, the hierarchical cluster analysis (H.C.A.), allows obtaining 5 groups of oocysts affiliated or not to the three farms Group1 comprises the majority of *Eimeria* species, first those that were not counted in the three sites: *E. intestinalis, E. piriformis, E. perforans, E. roobroucki* and *E. exigua***.** These 5 species form one cluster of points between the three stations and almost at the intersection linking the two axes (Fig. 2). Within the same group, it is also noted that there is *E. magna* and *E. media*, (Fig. 2), two species observed in the three considered locations. The second cluster is one that involves two variables Tigzirt, (E.stiedae) that is to say Tigzirt station and *E.stiedae* coccidia. The third assembly is that of: Makouda associated with *E. irrisidua* (Fig. 2) where as the fourth one regarding Ouacifs, contains *E.vejdovskyi* and *E.flavescens* (Fig. 2).

Point 5 comprises one single species, *E. coecicola* (Fig. 2), equidistant from the two stations of Makouda and Tigzirt, which indicates the presence of this coccidian in both stations simultaneously on the one hand, and its distance from Ouacifs farm on the other hand.



- Fig. 1 - Sporulated oocysts of seven identified *Eimeria* species of the rabbit *Oryctolagus cuniculus* study sites in Kabylia , (Algeria)

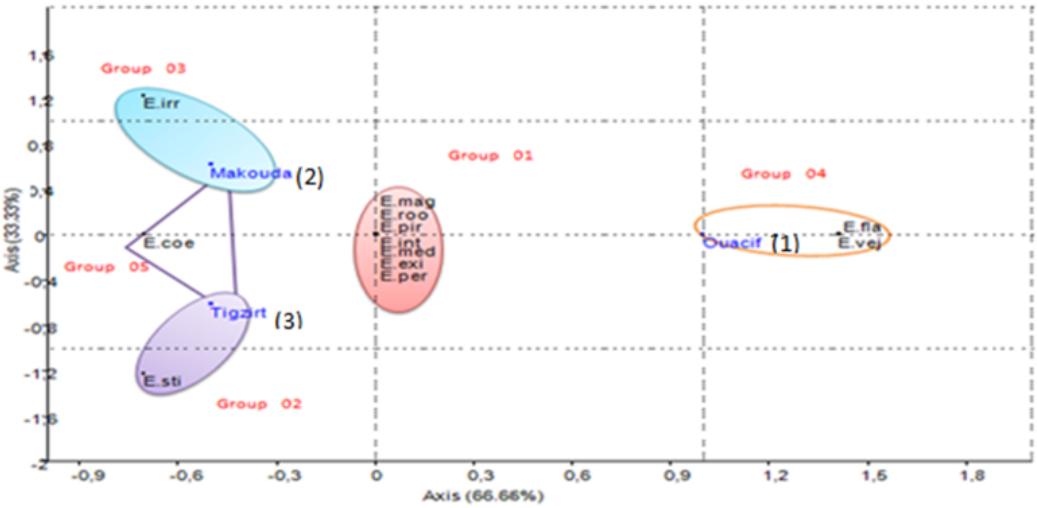
1 (a) and (b) *Eimeria media* , **2 (c)** *Eimeria flavescens* , **3 (d) and (e)** *Eimeria magna* **4 (f)** *Eimeria vejdovskyi* , **5 (g) and (h)** *Eimeria coeccicola* , **6 (i)** *Eimeria irrisidua*, **7 (j)** *Eimeria stiedae*
Scale bars=10 µm.

Table 1. - Morphological characteristics of identified *Eimeria* species found in the three breeding farms of domestic rabbits *Oryctolagus cuniculus* in Kabylia , Algeria measurements in µm

Oocysts	Shape	Residuum	Micropyle	Measurements Length x width	Scale of Residuum
Fig.1(a) and (b)	Ellipsoïd	Medium	Big	**(a)** 28 x 22 **(b)** 30 x 28	9 10
Fig. 1 (c)	Sherical	Absent	Wide	28 x26	Absent
Fig.1 (d) and (e)	Ellipsoïd	Big	Pronounced	**(d)** 30 x 28 **(e)** 30 x 25	12
Fig. 1(f)	Ellipsoïd	Small	Plate	**(f)** 36 x 18	5
Fig. 1(g) and (h)	Ovoïd elongated	Small relatively	Narrow	**(g)** 22 x12 **(h)** 30 x15	6
Fig. 1(i)	Ellipsoïd	Absent	Wide	**(i)** 24 x 15	Absent
Fig. 1(j)	Ellipsoïd	Absent	Absent	**(j)** 25 x 14	Absent

- Table 2. - Absence and presence of the spieces of *Eimeria* in each breeding farms of domestic rabbits *Oryctolagus cuniculus* in Kabylia , Algeria.

Spieces / Farms ►	Ouacifs	Makouda	Tigzirt	Spieces / Farms ►	Ouacifs	Makouda	Tigzirt
E. stiedae	0	0	1	*E. irresidua*	0	1	0
E. vejdovskyi	1	0	0	*E. intestinalis*	0	0	0
E. flavescens	1	0	0	*E. roobroucki*	0	0	0
E. magna	1	1	1	*E. exigua*	0	0	0
E. media	1	1	1	*E. piriformis*	0	0	0
E. coecicola	0	1	1	*E. perforans*	0	0	0



- Fig. 2: - Assembly of *Eimeria* oocysts according to the studied breeding farms of domestic rabbits *Oryctolagus cuniculus* in Kabylia, (Past,ver.1.98)

E.irr:*E. irresidua* , **E.coe**: *E. coecicola* , **E.sti** : *E. stiedae* ,**E.mag**: *E. magna* , **E .roo**: *E. roobroucki* , **E.pir**: *E.piriformis* , **E.int**: *E. intestinalis* , **E.med** : *E. media* , **E.exi**: *E. exigua* , **E.per**: *E.perforans* , **E.fla**: *E. flavescens* **E.vej**: *E. vejdovskyi,*

DISCUSSION

Regarding diagnosis, seven species of sporulated oocysts are identified in 384 samples. The listed oocysts are described by many authors mainly by Pellerdy [8] in Brazil, Peeters & Geeroms [9] in Belgium, Eckert *and al.* [6], and by Coudert *and al.* [7], in France. However, some species defined by these researchers have not been found in this study. *E. roobroucki* which was described by Gres *and al.* [10], in France, must be added among *E. intestinalis* *E.exigua*, *E.piriformis* and *E.perforans*. The oocysts *E.media*, *E.magna*, *E.stiedae* and *E.coecicola* collected during this research are confirmed in a investigation made in the farms of Mitidja and in a study conducted in Kabylia and eastern Mitidja by Henneb & Aissi [2]. The results of this same work show a predominance of the intestinal species with an association of 2 to 4 *Eimeria* species in the three farms (Tab. 2); which confirm those of Coudert *et al*. [7] that show that the rabbit is often a carrier of several *Eimeria* species. As to the affinities and distribution of *Eimeria* species found in the different farms, it should be pointed out that the absence of *E. intestinalis*, *E. piriformis*, *E. perforans*, *E. roobroucki* and *E. exigua* supports the results obtained by Nebri & Benkacemi [1] in Mitidja and of Henneb & Aissi [2] in Kabylia. Yet *E. perforans* was reported by Nebri & Benkacemi [1] in Mitidja. The dominant species are *E. magna* and *E. media* (Tab. 2).These results are in accordance with those of Bouchere & Nouaille [11] and those of Abdel-Azeem & Al-Quraishy [12] in a similar survey done in Riadh, Saudi Arabia. It should also be noted that *E.stiedae* was only collected from Tigzirt livestock (Fig. 2). Al-Mathal [13] maintains that this coccidian is dependent on the geographical position of the livestock, Darwish & Golemansky [14] ; state that the infection caused by this sporozoan is of 4% in Syria. This species found only in this Kabylian coastal site, at low altitude, is also indicated by Henneb & Aissi [2] in Bordj Menaiel of a microclimate comparable to that of Tigzirt. The presence of *E. irrisidua* in Makouda can be explained by its affinity to the highland and also by the poor maintenance of the farm. *E. vejdovskyi* and *E. flavescens* are found only in Ouacifs, a station with a harsh climate and a rugged terrain.

It should be recalled that *E. vejdovskyi* is a species that was first described by Pakandl [3] in 1988 in Czechoslovakia, a country with a cold climate similar to Ouacifs one in Djurdjura. The determination was made on the basis of the oocysts' morphology having the sizes 32.88×19.19 microns against 36 × 18 microns that were noted in Djurdura livestock, conforming to Pakandl's [3] data. Given that *E. coecicola* is present both in Tigzirt and Makouda and is absent in Ouacifs, it is suggested that this species likes more temperate microclimatic conditions.

CONCLUSION

This work is undertaken to approve the very few researches related to a coccidia census of *Eimeria* genus, intestinal parasite of domestic rabbits in Algeria. This study allowed the identification of 7species of which *E. flavescens* appears to be highly pathogenic. Being as a new species for Djurdjura (Kabylia), it should be indicated that *Eimeria vejdovskyi* is found in climates with very cold winters. The second part of this work as certain that *E. media*, *E. magna* and *E. coecicola* seem to have no predilection area. However, *E. vejdovskyi* may be extremely demanding in low temperatures. *E. irresidua* and *E. stiedae*, would not have particular affinities when *E. flavescens* could be rampant in Kabylian rabbit farms and consequently damage the livestock of the region.

REFERENCES

[1]. Nebri R & Benkacemi S. (2012). Recensement des espèces de coccidies du genre *Eimeria* Parasite du lapin dans les élevages de la Mitidja. 23ème Forum, 21-24 mars 2012, Hammamet, p.37.

[2]. Henneb M., & Aissi M. (2013). Etude cinétique de l'excrétion oocystale chez la lapine et sa descendance et identification des différentes espèces de coccidies. 15èmes Journées Recherche Cunicole, 19-20 novembre 2013, Le Mans, : *221- 224*,2013

[3]. Pakandl M. (1988). Description of *Eimeria vejdovskyi* sp. n. and redescription of *Eimeria media* Kessel, 1929. *Folia Parasitol*. (Praha). 35(1):1-9,

[4]. Pakandl M. (2009). Coccidia of rabbit: a review. *Folia Parasitol.* 56(3): 153–166,

[5]. El-shahawi G.A., El-Fayoumi H.M. & Abdelhaleem H. M. (2012). Coccidiosis of domestic rabbit (*Oryctolagus cuniculus*) in Egypt: light microscopic study. *Parasitol. Res.*, 110 (1): 251–258.

[6]. Eckert J., Taylor M., Licois D., Coudert P., Catchapole J. & Bucklar H. (1995). Identification of *Eimeria and Isospora* Species and Srains. Morphological and biological characteristics. In: Biotechnology. Guidelines on Techniques in Coccidiosis Research. Ed. Eckert J., Braun R., Shirley M.W., Coudert P., Luxembourg: *European commission, 103-119,*

[7]. Coudert P., Licois D. & Drouet-Viard F. (1995). *Eimeria and Isospora. Eimeria* species and strains of rabbits, in Biotechnology. Guidelines on Techniques in Coccidiosis research. Ed. Eckert J., Braun R., Shirley M.W., Coudert P., Sc., Luxembourg: *European commission, 52-73,*

[8]. Pelledry L. (1974). *Mammalia-Lagomorpha in Coccidia and coccidiosis*. Ed. Verlag, Paul Parey, Berlin, p. 959.

[9]. Peeters J.E. & Geeroms R. (1981). Coccidiosis in rabbits: afield study. *Res. Vet. Sci.*, 30. 328-334.

[10]. Gres V., Marchandeau S. & Landau I. (2002). Description d'une nouvelle espèce *d'Eimeria* (Coccidia, Eimeridea) chez le lapin de garenne *Oryctolagus cuniculus* en France. *Zoosystema*, 24 (2): 203-207.

[11]. Boucher S. & Nouaille L. (1996**).** *Maladies des lapins*. Ed. France agricole, Paris, 256 p.

[12]. Abdel-Azeem A. & Al-Quraishy S. (2013). Prevalence of Coccidia (*Eimeria* spp.) infection in domestic rabbits,*Oryctolagus cuniculus*, in Riyadh, Saudi Arabia. *Pakistan J. Zool., vol.* 45 (5): 1329-1333.

[13]. Al-mathal E.M. (2008). Hepatic coccidiosis of the domestic rabbit *Oryctolagus cuniculus domesticus* L. in Saudi Arabia. *World J. Zool.*, (3): 30-35,

[14]. Darwish A.I. & Golemansky V. (1991). Coccidian Parasites (Coccidia: Eimeriidae) of domestic rabbits (*Oryctolagus cuniculus* L.) In: Syria. *Acta. Protozool*., 31, 209-216.