WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 2, 54-67 Research Article ISSN 2278 – 4357

ISOLATION AND CHARACTERIZATION OF EIMERIA SPECIES IN POULTRY USING CONVENTIONAL METHODS AND COCCIMORPH PROGRAM IN KHARTOUM STATE, SUDAN

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Article Received on
29 Nov. 2018,

Revised on 19 Dec. 2018,
Accepted on 09 Jan. 2019

DOI: 10.20959/wjpps20192-13097

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ABSTRACT

An outbreak of Coccidiosis has a very high negative and economic impact on the flock as well as for the poultry producer as treatment alone cannot prevent the economic losses. This study was carried out in Khartoum State during the year 2018. Samples for isolation of field Eimeria species' oocysts were collected from droppings and intestines of chickens from chicken flocks farms and Slaughter houses located in different localities of Khartoum State. Samples were taken from birds showing typical clinical signs of coccidiosis and not subjected for treatment. Eighty samples (45 faecal and 35 intestine samples) were collected aseptically from suspected birds from different localities of Khartoum State. Samples were subjected for parasitological and online Coccimorph identification system. Out of 80 samples collected from Khartoum State, 54 samples (67.5%) were positive for Eimeria Oocyst. Out of 50 samples collected from open rearing system, 33 samples (66%) were positive for Eimeria Oocyst. Out of 30 samples collected from closed rearing system, 21 samples (70%) were positive for Eimeria Oocyst. Out of 35 faecal samples collected for Eimeria Oocyst isolation, 28 samples (80%) were positive for Eimeria Oocyst. Out of 45 intestine samples collected for Eimeria Oocyst isolation, 26 samples (57.8%) were positive for Eimeria Oocyst. Out of 54 Eimeria Oocyst isolated from Khartoum State and subjected to convential methods and Coccimorph program

characterization, the identified Eimeria spp. were: 21 *Eimeria mitis* (38.8%), 19 *Eimeria necatrex* (35.2%), 7 *Eimeria tenella* (13%) and 7*Eimeria maxima* (13%). This is the first report in Sudan of using Coccimorph system for identification of *Eimeria spp.* The isolates scored high probability percentages.

I. INTRODUCTION

Avian coccidiosis is a parasitic disease of intestinal tract caused by single cell protozoan parasite belonging to the genus Eimeria. In the domestic fowl, seven Eimeria spp. are recognized: *E. brunettie, E. maxima, E. necatrix and E. tenella* are highly pathogenic, while *E. acervulina* and *E. mitis* are rather less pathogenic and *E. praecox* is regarded as the least pathogenic.[1] Coccidiosis has been considered as a very harmful disease affecting growth and performance of birds in the intense of poultry[1] [2] [3] it is contributory factor in the pathogenesis of several diseases.[4][5] According to a recent estimate by[6], coccidiosis may cost the US chicken industry about $127 million annually and likewise similar losses may occur worldwide. Thus coccidiosis is probably the most expensive and wide spread infectious disease in commercial poultry production systems. Coccidiosis causes massive destruction of the epithelial cells, which leads to bloody diarrhea, reduced weight gain and temporary reduction in egg production.[7] Eimeria spp. is omnipresent and can survive in infected birds and the external environment for long times.[8] It causes high mortality in young chicks because most of the Eimeria spp. affects birds between the age of 3 and 18 weeks.[9] The accurate identification of Eimeria species is essential in control and treatment of poultry coccidiosis. The disease is endemic in most of the tropical and subtropical regions where ecological and management conditions favour an all-year round development and propagation of the causal agent.[10] [11] In the Sudan five Eimeria species were identified during an outbreak of coccidiosis in farms at Khartoum. These included: *E. tenella E.maxima, E. mivati, E. praecox and E. brunette.*[12] Babiker *et al.;* 2009) reported that coccidiosis caused mortality in poultry farms in Khartoum State. Also[12] reported two spp. Include *Eimeria tenlla and Eimeria acervulena* in Khartoum State. This study was carried out in Khartoum state which represents the political, economic and cultural capital of Sudan. The study covered different farms in different localities of Khartoum State.

This study was Aiming for identifying *Eimeria* spp. implicated in poultry coccidiosis in Khartoum State.

II. MATERIALS AND METHODS

2.1. Source of samples

Samples for isolation of field Eimeria species' oocysts were collected from droppings and intestines of chickens from chicken flocks farms and slaughterhouses located in different localities of Khartoum State. Samples were taken from birds showing typical clinical signs of coccidiosis and not subjected for treatment.

Eighty samples (45 faecal and 35 intestine samples) were collected aseptically from suspected birds from different localities of Khartoum State.

2.2.2. Sampling procedure

Samples were labeled by writing the name of farm, date and address, brought in ice boxes to the Faculty of Veterinary Medicine, University of Bahri and were kept at 4C˚till examination and identification of coccidian oocysts.

2.3. Parasitological Examination

2.3.1. Examination of intestine samples

The intestine of slaughtered chickens was placed in a tray. Double ligature was applied to separate different parts of the intestine into: duodenum, jejunum, ileum and cecum. Each part of the intestines was opened by scissor giving longitudinal incision and its contents were collected in a beaker. Contents were examined by direct microscopic smear and standard floatation method for the presence of Eimeria spp. Oocysts.[13] Saturated sodium chloride (NaCl) solution was used for flotation technique to isolate Oocysts.[14] The aspirated mixture was diluted with distilled water (4:1) to remove sodium chloride. The sediment obtained was subjected to sporulation. The contents from positive samples were placed in 2.5 per cent potassium dichromate (K2Cr2O7) solution. Eimeria oocysts were purified and identified according to size and shape after sporulation, sporulation time and Coccimorph software (http://www.coccidia.icb .usp.br/coccimorph/).[15]

2.3.2. Examination of the faecal samples

The faecal samples were soaked overnight at 37°C in 2.5% (w/v) aqueous solution of potassium dichromate. The samples were shaken vigorously to break up the faeces. The suspension was filtered through a cheese cloth into a beaker. Saturated sodium chloride (NaCl) solution was used for flotation technique to isolate oocysts.[14] The filtrate obtained was centrifuged at 2000 rpm for 5-10 minutes to settle down the Oocysts. The supernatant

fluid was discarded and the Eimeria Oocysts present in the sediment were separated using floatation technique and then examined carefully through microscope using oil emulsion lens for the presence of the Eimeria oocysts. Photographs of the positive slides were taken. Direct microscopic examination a pin head drop of faecal was put on a microscopic slide, mixed well with a drop of saline 0.9% by the aid of a wooden stick, covered with a cover glass slip and examined under high power X40 of light microscopic for detection of any Oocyst in faeces.

2.3.3. Sporulation of Oocysts

The aspirated mixture was diluted with distilled water (4:1) to remove sodium chloride. The washed samples were placed in 2.5% potassium dichromate (K2Cr2O7) solution in petri dishes. The petri dishes were partially covered to allow the passage of oxygen and were incubated at 37C˚for 48 hours.[16] The contents of petri dishes were stirred off and on to ensure the oxygenation of the oocyst. During sporulation 60-80% humidity was maintained by placing tow petri dishes containing water in the incubator. The sporulation of the oocyst was confirmed by taking a drop of the mixture and examined for the sporocysts under the microscope.

2.3.3.1. Purification of sporulated oocysts

The suspension having the sporulated oocysts was mixed thoroughly with equal quantity of 2.5% potassium dichromate (K2Cr2O7) solution to acculturate sporulation. The suspension was then filtered through a sieve followed by muslin cloth and centrifuged at 1500 rpm for 10 minutes. Almost 50% of the supernatant was discarded and the remaining portion of the supernatant in the centrifuge tubes was poured in a fresh tube and mixed with an equal quantity of saturated sodium chloride (NaCl) solution for flotation.[17] The supernatant having sufficient number of sporulated oocysts was aspirated by pipetting system and collected separately in a tube. The sediment was processed in the same way until no sporulated oocysts remain in the supernatant. The supernatant thus collected was mixed with water (1:5) in a falcon tube and kept undisturbed overnight at 4ºC. The sporulated Oocysts settled in the bottom were collected by removing all water through suction by pipetting system. The supernatant was removed and the remaining mixture at the bottom, having the sporulated Oocyst. Oocysts were examined by measuring their length and width with light microscopy, armed with calibrated ocular lens as well as determination of the oocysts shape and length/width index).[13]

2.3.3.2. Oocyst and shape index

Oocyst shape index (Length / Width) was determined by measuring its length and width using a calibirated oacular micrometer at X40 magnification (Fig.3) the likely Eimeria species contained in each sample were determined by comparing the calculated oocysts with the guides provided by.[18] Oocysts also determined according to.[13]

2.4. Identification of *Eimeria* spp. by Coccimorph software[19]

2.4.1. Diagnosis of oocysts through digital image processing

For identification of *Eimerian* oocysts, photomicrographs of sporulated oocysts were taken from each sample at 10×/40× using a dry high power objective with a photomicrographic camera (Moticam5, Hong Kong) attached to a trinocular research microscope (Motic Trinocular Research Microscope BA210, Hong Kong). The identification of *Eimeria* spp. of chickens was done using COCCIMORPH software (http://www.coccidia.icb .usp.br/coccimorph/). The software was downloaded from the Internet and the oocyst images (400×magnification) were uploaded for species identification as described online (Fig.1). The *Eimeria* spp. identified by the software in each sample was recorded.

2.4.2. Capturing images

1. The image was captured at the highest resolution, regularly used for 4- megapixel camera with a 40x magnification objective. The lower resolution was used, the less information was provided for COCCIMORPH. In our experience, images with less than 2 megapixels were hardly diagnosed.

2. Crop out single oocyst images to use in the program.

3. Always use well-defined oocysts images, especially well focused images.

4 Upload image file was used TIF, JPEG, BMP or PNG file formats. Do not used GIF format, as it results in a severe loss of information, thus decreasing the accuracy of COCCIMORPH.

5. The scale value was determined for capture equipment and fill in the Scale box in COCCIMORPH's interface.

6. Clicked on the segment image button and was used the arrow buttons, find the most adequate threshold value for segmentation.

7. The observation of the results for classify button was clicked.

2.4.3. Online diagnosis procedure

The online diagnosis system is located at http://puma.icb.usp.br/coccimorph. Or http://puma.icb.usp.br/uploadoocyst/uploadimg.php**I**

A. The Scale box, was the filled in the pixels/micrometer value of capture system for scale normalization.

B. Segmentation threshold section. This value was defined the threshold for the system to determine the object contour. The lateral buttons (<<, >, >, >>) permit to change the value in one- or five-unit increments, respectively. To monitor the effect of the new segmentation value, the user must press the Segment image button.

C. The Select image file box. The full path of the image file was specified here. Alternatively, was browsing the disk and select the file by clicking on the Browse… button.

D. The image file was selected and uploaded by pressing the Upload image button.

E. The image was classified process executed by clicking the Classify button. Typical result was displayed in the figure (16).

F. The results were sent by e-mail.

II- RESULTS

3.1. Epidemiology of broilers' coccidiosis in Khartoum State

3.1.1. Areas of samples collected

Eighty samples (45 gut and 35 faecal samples) were collected aseptically from infested broilers from different localities of Khartoum State.

3.2. Positive samples for Eimeri Oocyst in Khartoum State

Out of 80 samples collected from Khartoum State, 54 samples (67.5%) were positive for Eimeria Oocyst.

3.2.1. Broilers' coccidiosis in different rearing systems Khartoum State

Out of 50 samples collected from open rearing system, 33 samples (66%) were positive for Eimeria Oocyst. Out of 30 samples collected from closed rearing system, 21 samples (70%) were positive for Eimeria Oocyst (Table 1).

3.2.2. Numbers and percentage of Eimeria Oocyst isolated from intestine and faecal sample in Khartoum State

Out of 35 faecal samples collected for Eimeria Oocyst isolation, 28 samples (80%) were positive for Eimeria Oocyst. Out of 45 intestine samples collected for Eimeria Oocyst isolation, 26 samples (57.8%) were positive for Eimeria Oocyst (Table 2).

3.2.3. Eimeria spp. isolated from Khartoum State

Out of 54 Eimeria Oocyst isolated from Khartoum State and subjected to convential methods and Coccimorph program characterization, the identified Eimeria spp. were: 21 *Eimeria mitis* (38.8%), 19 *Eimeria necatrex* (35.2%), 7 *Eimeria tenella* (13%) and 7*Eimeria maxima* (13%). (Table 4 and Figures 2, 3, 4, 5 and 6).

Table (1): Numbers and percentage of Eimeria Oocyst isolated from different rearing systems in Khartoum State.

Open system		Closed system		Total
No. of Samples	No. and % of positive Samples	No. of samples	No. and % of positive samples	
50	33(66%)	30	21 (70%)	80

Table (2): Numbers and percentage of Eimeria Oocyst isolated from intestine and faecal sample in Khartoum State.

Intestinal samples		Faecal samples		Total
No. of Samples	No. and % of positive Samples	No. of samples	No. and % of positive samples	
45	26 (57.8%)	35	28 (80%)	80

Table (3): Eimeria spp. oocysts' morphology, shape index of result and shape index of Soulsby**.**

Eimeris spp.	Shape Index (µm)		Oocysts' Morphology
	Result	Soulsby	
E. mitis	1 to 11	1.13 to 1.4	sub-spherical
E.necatrex	1.2 to 1.23	1.13 to 1.4	ovidal
E.tenella	1.24 to 1.3	1.195 to 1.32	ovidal
E.maxima	1.4 to 1.5	1.26 to 1.38	ovidal

Table (4): Eimeria spp. isolated from Khartoum State.

E. mitis	*E. necatrix*	*E. tenella*	*E.maxima*	Total
21(38.8%)	19(35.2%)	7(13%)	7(13%)	54(100%)

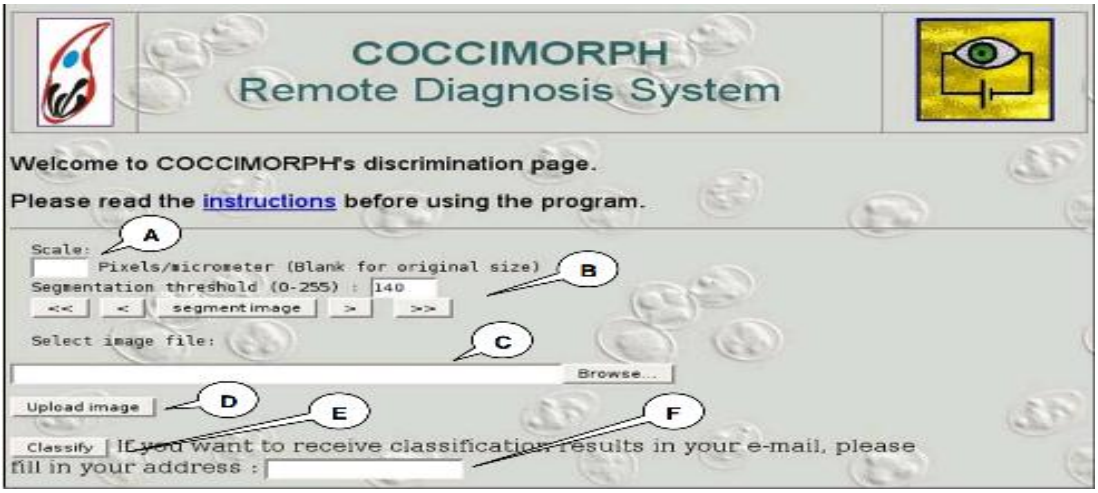

Fig. (1): Web site page of Coccimorph Remote Diagnosis System of Eimeria spp.

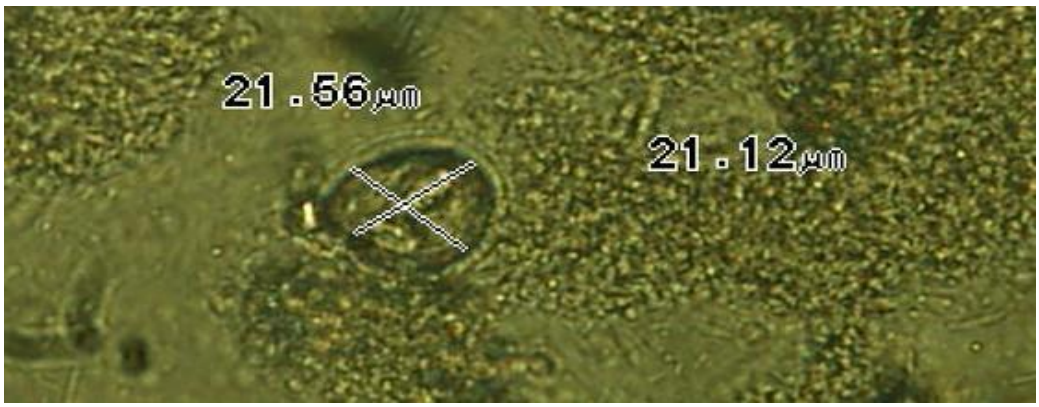

Fig. (2): *E. mitis Oocyst's* dimentions.

Fig. (3): *E. necatrix Oocys'* dimentions.

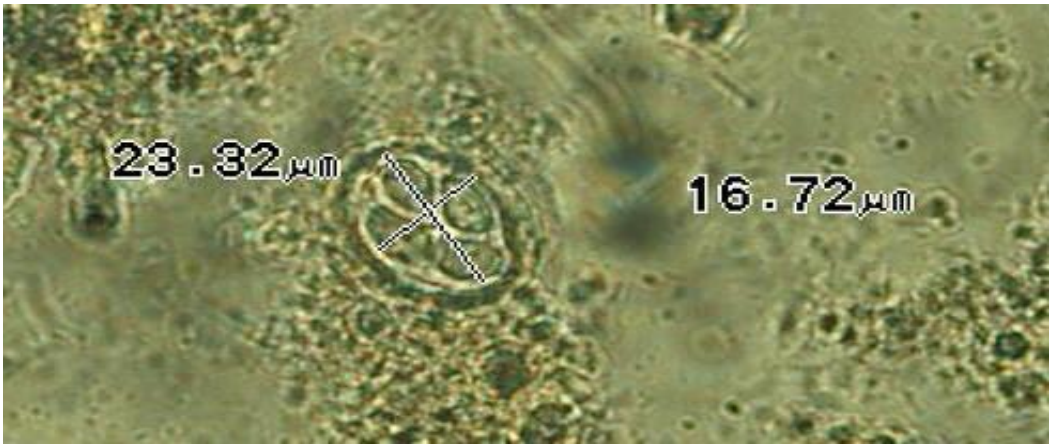

Fig. (4): *E. maxima Oocyst's dimentions.*

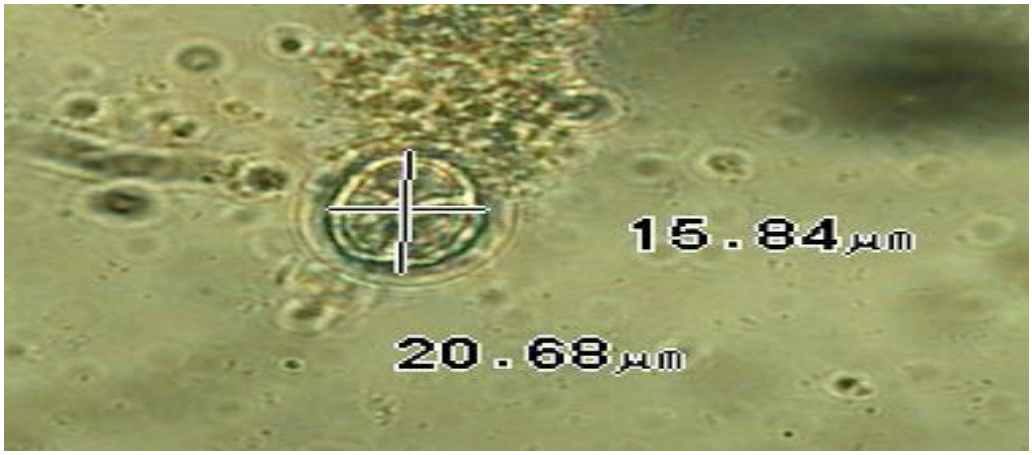

Fig. (5): *E. tenella* Oocyst's dimentions.

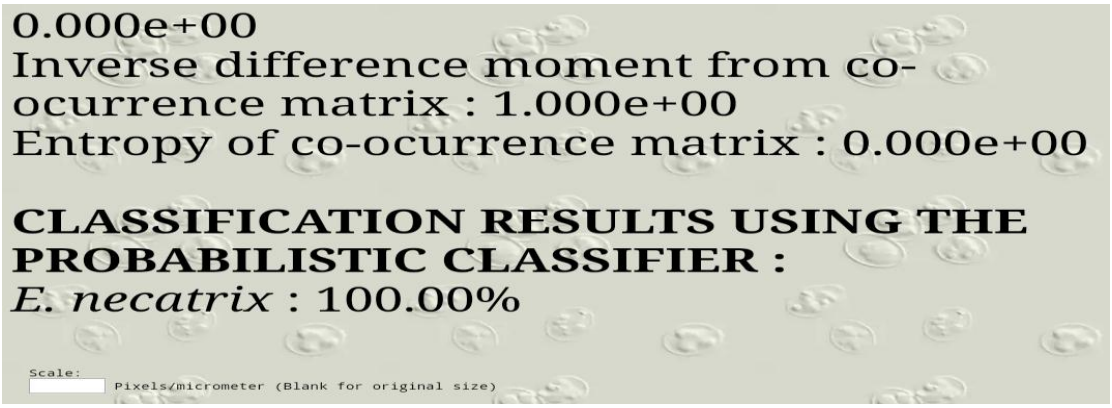

Fig. (6): Screenshot of final classification report of oocyst.

IV. DISCUSSION

Coccidia are almost universally found wherever chickens are raised. It was exceedingly rare to find any commercial chicken flock not affected by Eimeria.[20] Their strict host specificity eliminates wild birds as sources of infection. The most common means of spread of coccidia

is mechanical, by personnel that move between pens, houses, or farms. Coccidial infections are self-limiting and depend largely on the number of oocysts ingested and on the immune status of the bird.[21] This study aimed at identifying Eimeria spp. implicated in poultry coccidiosis in Khartoum State. 80 samples (45 gut and 35 faecal samples) were collected aseptically from broilers showing typical clinical signs of coccidiosis and were not subjected for medical treatment, from different localities of Khartoum State. Cases of broilers' coccidiosis in open rearing system represented 33 (66%) compared to cases in closed system 21 (70%).[22] also reported a high occurrence (53.6%) of coccidiosis in open system in Gammu region compared with closed system (25.6%). Cases of broilers' coccidiosis represented 12 (60%) in Khartoum locality, (72%) in Omdurman locality and 24 (68.5%) in Bahri locality and this may be due to large poultry population in Bahri locality than Omdurman and Khartoum localities. The study disagrees with[14] who reported a low prevalence 4.4% and 6.7% of Eimeria spp. in open and closed systems, respectively in Some Broiler Farms in Khartoum State, Sudan. In the present study, the mean values of *E. mitis* oocysts measurement were (1 to 1.1 µm), *E. necatrex oocysts* (1.2 to 1.23µm), *E. tenella* oocysts (1.24 to 1.3µm) and *E. maxima* oocysts (1.4 to 1.5) shape index. These findings are in agreement with those of[13] for the same species, with[23] in *E. necatrex*, but disagreement in *E. tenella.* As well in agreement with[12] in *E. tenella.* 54 Eimeria spp. were isolated from Khartoum State. The isolated Eimeria spp. represented: 21 *E. mitis* (38.9%), 19 *E. necatrex* (35.1%), 7 *E. tenella* (13.0%) and 7 *E. maxima* (13.0%).[1] mentioned that in domesticated chickens, at least nine species of Eimeria have been recognized : *E.brunette, E. maxima, E. necatrix and E. tenella* are highly pathogenic, *E. acervulina, E. mitis and E. mivati* are rather less pathogenic, while *E. praecox* and *E. hagani* are regarded as the least pathogenic. 33 (66.0%) Eimeria spp. were isolated from 50 samples collected from open rearing system and 21(70%) Eimeria spp. were isolated from 30 samples collected from closed rearing system. Out of 54 Eimeria spp. isolated from Khartoum State, 33 (66.0%) Eimeria spp. were isolated from open broilers' rearing systems and 21(70%) from closed system. These findings agree with those[24] who reported that the prevalence of coccidiosis in broiler chickens in District Dera Ismail Khan, Pakistan was 41.51% and among young broiler chickens was 65.96%. The isolated Eimeria spp. represented 12 (60%) in Khartoum locality, 18 (72%) in Omdurman locality and 24 (68.5%) in Bahri locality. This study disagrees with[14] who reported low prevalence 10%, 6.7% and 0% of Eimeria spp. in Khartoum, Khartoum North and Omdurman, respectively. The isolated Eimeria spp. from Khartoum State represented: 21 *E. mitis* (38.9%), 19 *E. necatrex* (35.1%), 7 *E. tenella* (13.0%) and 7 *E. maxima* (13.0%). The

study disagrees with[25] who isolated *E. tenella* (61.5%), *E. maxima* (12%), and *E. acervulina* (1.5%) in Tunisia. And disagree with[26] who reported that *E. tenella* was the dominant species with an infection rate of 5.5% followed by *E. acervulina* 2.2%. Twelve Eimeria spp. were isolated from Khartoum locality, the isolates were: 6 *E. mitis* (50.0%), 4 *E.necatrex* (33.4%) 1 *E.tenella* (8.3%) and 1 *E. maxima* (8.3%). 18 Eimeria spp. were isolated from Omdurman locality. The isolates were: 6 *E. mitis* (33.3%), 4 *E. necatrex* (22.2%), 3 *E. tenella* (16.7%) and 5 *E. maxima* (27.8%). 24 Eimeria spp.were isolated from Bahri locality. The isolates were: 9 *E. mitis* (37.5%), 11 *E. necatrex* (45.8%), 3 *E. tenella* (12.5%) and 1 *E. maxima* (4.2%). The finding agrees with [24] who isolated four species of *Eimeria; E. tenella* (24.24%), *E. maxima* (31.06%), *E. mitis* (31.82%) and *E. necatrix* (12.88%) were observed. Also the findings agrees with[22] who reported prevalences of 90%, 88%, 72%, 68%, 60%, 26%, and 8% for *E. tenella*, *E. praecox*, *E. acervulina*, *E. maxima*, *E. mitis*, *E. necatrix*, and *E. brunetti*, respectively. This indicates that *E. tenella*, *E. praecox*, *E. acervulina*, *E. maxima*, and *E. mitis* are the predominant species. Eimeria spp. isolated from intestine samples in Khartoum State represented 26 (57.7%) and Eimeria spp. isolated from faecal samples represented 28 (80%) out of 54 isolated. The finding goes in line with those of[27] that Eimeria spp. could be isolated from faecal samples. The same *Eimeria spp.* were identified using conventional method and computerized method (coccimorph software). According to interpretation of oocysts, isolates scored high probability percentages. With success against limitations Coccimorph could be replaced for manual conventional methods in clinical lab. Economic studies estimate a good future for Coccimorph system in Parasitology lab.[27]

V. CONCLUSION

This study concluded the followings:

1- Broilers' coccidiosis in open rearing system represented 50 (62.5%) compared to cases in closed system 30 (37.5%).

2- Broilers' coccidiosis represented 20 (25.0%) in Khartoum locality, 25 (31.2%) in Omdurman locality and 35 (43.8%) in Bahri locality of Khartoum State.

3- Thirty three (66.0%) Eimeria spp. were isolated from 50 samples collected from open rearing system and 21 (70%) Eimeria spp. were isolated from 30 samples collected from closed rearing system (Table 4). Out of 54 Eimeria spp. isolated from Khartoum State, 33 (66%) Eimeria spp. were isolated from open broilers' rearing systems and 21(70%) from closed system.

4- The isolated Eimeria spp. represented 12 (22.2%) in Khartoum locality, 18 (33.3%) in Omdurman locality and 24 (44.5%) in Bahri locality.

5- The isolated Eimeria spp. from Khartoum State represented: 21 *E. mitis* (38.9%), 19 *E. necatrex* (35.1%), 7 *E. tenella* (13.0%) and 7 *E. maxima* (13.0%).

6- Twelve Eimeria spp. were isolated from Khartoum locality, the isolates were: 6 *E. mitis* (50.0%), 4 *E.necatrex* (33.4%) 1 *E.tenella* (8.3%) and 1 *E. maxima* (8.3%). 18 Eimeria spp. were isolated from Omdurman locality. The isolates were: 6 *E. mitis* (33.3%), 4 *E. necatrex* (22.2%), 3 *E. tenella* (16.7%) and 5 *E. maxima* (27.8%). 24 Eimeria spp.were *isolated* from Bahri locality. The isolates were: 9 *E. mitis* (37.5%), 11 *E. necatrex* (45.8%), 3 *E. tenella* (12.5%) and 1 *E. maxima* (4.2%).

7- Eimeria spp. isolated from intestine samples in Khartoum State represented 26 (48.1%) and *Eimeria spp.* isolated from faecal samples represented 28 (51.9%) out of 54 isolates.

8- This is the first report in Sudan of using Coccimorph system for identification of *Eimeria spp.* The isolates scored high probability percentages.

VI. ACKNOWLEDGEMENT

Authors are thankful to University of Bahri for providing necessary facilities to carry out this research work.

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