RESEARCH ARTICLE

Prepare Local Vaccine for Vaccination of Chicken Against Coccidiosis by using Live Oocysts of Different Species of Eimeria

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ABSTRACT

The study was conducted to vaccinate chickens against coccidiosis by using a live vaccine containing 7 species of chicken Eimeria, Eimeria *tenella* (40%), Eimeria *brunetti* (21%), Eimeria *necatrix* (20%), Eimeria *maxima* (7%), Eimeria *mevati* (6%), Eimeria *acervulina* (3%), Eimeria *praecox* (3%). A total of 120 chicks at one day of age, Broilers Habbard type used in the Experiment. The birds of the Experiment were allocated on 3 pins; each contains 40 chicks.

The birds chosen for the Experiment were vaccinated at the 9th day of age, with a suspension of mixed Eimeria that contain 50 oocysts of *E. tenella*with percent of other species, respectively. The vaccine was given to chicks in the 1st pins (40 chicks) with drinking water. The chicks in the 2nd pin were given vaccine by crop inoculation, the chicks in the 3rd pin left non-vaccinated as a control group. All groups were challenged at 25 days of age with 50000 sporulated oocysts of live *E. tenella*with the percentage of other species found in the suspension by crop inoculation. The mortality % was recorded daily till the end of the Experiment. Clinical signs observed 4 to 10 days post-vaccination and challenged. Body weight was measured at 25, 32, 48 days of age, and weight gain was calculated. Fecal sample collected at 16, 25, 32 days of age and examined for oocysts count. The following parameters had been done seven days post-vaccination and challenge: Packed cell volume (PCV), score lesion of intestine and caecum.

The experiment results pointed out that the vaccinated chicks develop resistance against the Eimeria species present in the suspension; by reducing in the clinical signs of vaccinated groups seven days post-challenge. The Experiment was recorded a significant difference between vaccinated and non-vaccinated in mortality percentage; control groups recorded the highest rate of mortality compared with vaccinated groups. The PCV elevated significantly in vaccinated groups after challenge compared with that of the control group. The chicks showed significantly highest weight gain, in the control group and bird vaccinated in drinking water, comparing with those of the crop inoculation group.

Seven days post-challenge, lesion scoring of intestine and caecum were reduced significantly in vaccinated groups compared with control. In the chicks' feces, the oocysts count showed a significant reduction in the number of oocysts in vaccinated groups compared to control groups 7 days post-challenge. The results showed no significant differences in all parameters measured in the experiments between the birds that were vaccinated with drinking water or by crop inoculation. The protection ratio in broiler arranged between, 89-90% experiment respectively, depending on the method of inoculation. It was concluded that the prepared vaccine, which was given to the chicks at 9 days of age decreases the severity of the clinical signs, mortality percentage, lesion scours, and oocysts count in the feces of vaccinated broilers.

Keywords: Coccidiosis, Eimeria, Parasites, Vaccination.

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INTRODUCTION

Coccidiosis is an important disease that is widespread in all parts of the world, caused by parasitic protozoans that affect the digestive system of poultry and all kinds of animals and humans except for the equine species.[1] Despite the progress made in treatment and prevention, the disease still causes significant economic losses represented in the high rate of mortality.[2] As well as low weight and increased susceptibility to secondary infections, bacterial and viral.[3]

Coccidiosis caused a persistent problem in the poultry industry, and the recurrence of infection became an economic problem in the fields of poultry raising due to the continuous occurrence of drug resistance.[4] Since the beginning of the last century, many researchers have carried out numerous studies to limit the disease's spread. Some of them have focused on the aspect of education and good management.[5] Others were most concerned with the quality of feed provided for spawning, such as the addition of some substances.[6] Some people used anti-coccidiosis.[7] Others, along with immunity, also gave great importance to controlling the disease, and several immunization programs were followed, including natural exposure to the disease with medications[8] or the extraction of inanimate materials for the parasite as a vaccine[9] or use weakened Eimeria oocysts in different ways[10] or the use of fierce Eimeria oocysts in a few numbers.[11]

In recent years, there have been high levels of coccidiosis infection, which means that the same bird will be infected with several emery types. Due to the severity of some cases, they cause great economic losses due to declines and lack of productivity, so many researchers have tended to find vaccines to limit the disease's spread. This, in turn, developed many coccidiosis mixed vaccines for chickens, and the use of a small number of Eimeria oocysts to immunize against infections with coccidiosis is considered a good method that has dimensions that many animal breeders aspire to, as it works to create natural immunity with high efficiency, and easy to use In addition to the ease of manufacturing at minimal costs, compared to the vaccination by irritated weakened Eimeria oocysts[12] because live Eimeria oocysts are good antigens for high immune response events[13] without affecting the consumer[11] as emphasized by Allen P C, *et al*.,[14] and Hassan A A, *et al*.,[15] that live vaccine has proven to be a good efficiency in protecting chicks from coccidiosis infection, as it reduced mortality and severity of lesions; the numbers of Oocysts in the bed, and scored better results than the use of coccidiosis salinomycin. The current study focuses on this type of immunization, choosing the lowest doses, and obtaining adequate protection without causing damage to be applied in the field. The research objectives included conducting this study to know the extent of the possibility of vaccination with a local vaccine containing seven types of Eimeria spread in Iraq, which contain a small number of parasitic oocysts Eimeria living and know the percentage of protection without affecting the chicks.

MATERIALS AND METHODS

A total of 120 Hubbard chicken, 1-day old, were bred in a large hall with plastic barriers. The hall was equipped with lamps that continuously operated to control the temperature using gas incubators. Sterile water and fodder were provided for chicks. All vaccines were given except for coccidiosis. Eimeria Oocysts used for immunization were prepared, purified, and infected with the challenge dose by collecting the intestines of chickens infected with different types of Eimeria.

Preparing, Purifying, and Sterilizing the Eimeria Oocysts

The mucosa and vessels were removed with the epithelial layer using a glass slide; then, the contents were collected in clean glass bottles containing 2.5% potassium dichromate and for purification of impurities placed in a small filter (60 net/inch). The solution was placed in Petri dishes and left at 28°C for 72 hours to accelerate the sporulation process. The Oocysts were washed with distilled water three times after sedimentation by placing them in the centrifuge for 5 minutes at a speed of 2000 rounds/min. After removing the floating solution in the third stage, a saturated saline solution was added to the precipitate for the purpose of floating. The floating solution was withdrawn and placed in a glass jar; then, the solution was diluted with distilled water in a ratio of 1:10. The Oocysts were then deposited by placing them in a centrifuge then the precipitate was collected in clean bottles. Eimeria parasite Oocysts used in the experime were sterilized according to the specific density, according to the method.[16] Relative to Eimeria Oocysts spore using the Haemocytometer Egg Blood Cells Calculation Method.[17,18]

Calculating the Percentage of Oocysts Types in Isolates used in the Experiment

The percentage of sporulated Eimeria oocysts in isolates was calculated according to the differential number of white blood cells. By calculating 100 Eimeria oocysts and separating them into the ones in isolation, then the percentage for each species was calculated. This process was repeated 5 times, after which the percentage of Eimeria oocysts types were calculated in isolates used for the purpose of vaccination and challenge. The percentage of each species in the suspended solution was as shown in Figure 1.

Experiment Design

The Experiment included fortifying the broiler chicks with the prepared solution, containing seven different types of Eimeria parasites, containing 50 *E. tenella* Oocysts with different proportions of other types found in the suspended solution (Table 1).

Total Number of Chicks used in the Experiment: A total of 120 chicks were divided equally into three cages. The chicks were immunized in the first and second cages by dosing the birds by pumping the microbial preparation directly into the chicken crop and drinking water, respectively. The Group 3 was left without immunization (control group).

Notes

- All vaccines were given to all chicks except for antacids.
- The number of deaths was calculated daily.

Eimeria praecox	*Eimeria acervulina*	*Eimeria mevati*	*Eimeria maxima*	*Eimeria necatrix*	*Eimeria brunette*	*Eimeria tenella*
3%	3%	6%	7%	20%	21%	40%

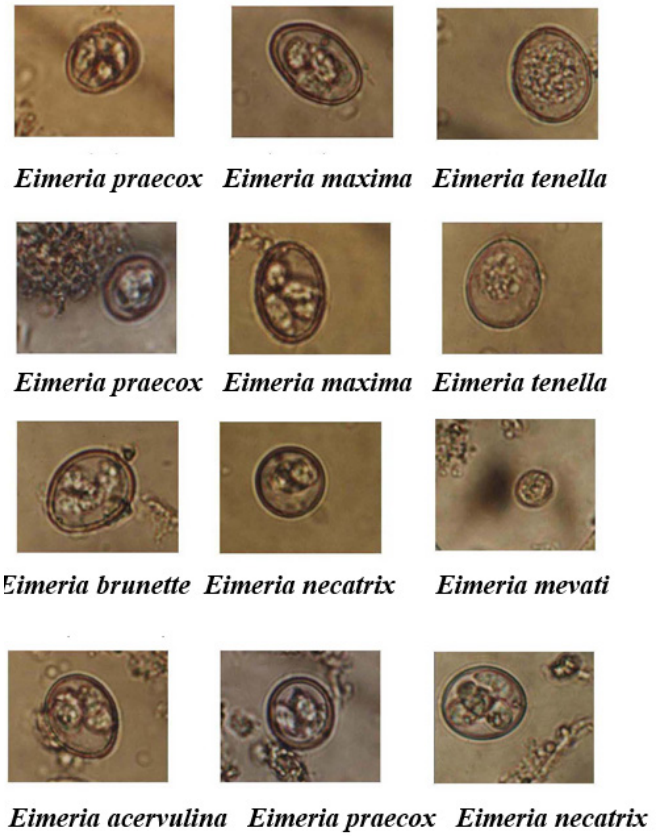

Figure 1: Eimeria oocysts types under study

The Parameters Used in the Experiments

- *Clinical Signs:* Clinical signs were observed in chicks after the fourth day of vaccination and after the challenge dose until the tenth day.
- *Mortality:* Mortality was recorded daily for all tests, and the percentage of deaths after the dissection of dead chicks was calculated using the following formula:[20]

  Mortality percentage = [The number of dead chicks/The total number of chicks] × 100
- *Measured the Percentage of Packed Red Blood Cells Volume:* The percentage of packed red blood cell volume was measured according to the method.[19]
- *Calculating the Weight Gain:* All individual chicks in all tests with a sensitive balance after immunization as well as after the challenge and at the end of the chicks breeding period for the purpose of calculating the weight gain; the weight gain was calculated based on the following formula:[20]

  Weight gain = body weight at the end of the period – live body weight at the beginning of the period in grams
- *Estimating the Severity of the Lesions Visually:* The severity of the lesions was visualized according to the method.[20]
- *Calculation of the sporulated E. tenella Oocysts in Guano:* The modified McMaster method was used,[21] and the following equation was used to calculate the number of Oocysts in grams of Guano:

  Eimeria oocysts number in one gram of Guano = (The number of oocysts in one hall × 15) / 0.15
- *Calculation of the Percentage of Protection:* The percentage of protection generated by the vaccinated chicks was calculated by calculating the Eimeria oocysts number in one gram of Guano and the non-immunized chicks (control group) after seven days of performing the

Table 1: Experiment design

Days	*Experiment*
9	The chicks were given in the first group, the vaccine prepared with the suspended solution by crop, and in the second group with drinking water, then the third group was left without the vaccine as a control group.
12	Clinical signs of the disease are observed daily from the fourth day until the tenth day after immunization.
13	All chicks were vaccinated with Newcastle + the first Gumboro vaccine.
16	Six chicks were randomly selected from each test to calculate the following criteria: measuring packed red blood cell volume, calculating the Oocysts in Guano, then chicks' dissection to estimate the severity of macroscopic lesions (on the seventh day after vaccination).
19	All chicks were vaccinated in all tests with the second Newcastle vaccine.
21	All chicks were vaccinated in all tests with the second Gumboro vaccine.
25	All individual chicks were weighed to calculate the weight gain. Six Guano samples were collected from each chicken coop to detect the presence of Eimeria oocysts. Then, all chicks were given in all tests the Challenge doses containing 50000 sporulated *E. tenella*Oocysts with the rest of the Eimeria species according to the proportions in the suspended solution by dosing in the crop.
28	Clinical signs of the disease are observed daily from the fourth day until the 10th day.
32	All chicks were individually weighed for the purpose of calculating the weight gain Then the criteria that were calculated on the 16th day of life (on the seventh day after the challenge) were recalculated.
36	All the chicks were given in all tests, the third new castle vaccine with the anti-mycoplasma + vitamins A, D3, E
48	All individual chicks were weighed to calculate the weight gain.

challenge examination using the method[22] and according to the following formula:

Protection ratio % = [(The average number of Oocysts / gram of Guano for control group) – (The average number of Oocysts / Gram of Guano for vaccinated group) / (The average number of oocysts/gram for the control group)] × 100

Statistical Analysis

The statistical analysis of this experiment data was performed using the ANOVA variance analysis method. To differentiate between treatments, least significant different (LSD) was used. To find the statistical significance level for the differences between the rates, the percentage of the volume of plasma red blood cells, the weight gain calculation, the intensity of macroscopic lesions, the number of Oocysts presented with the droppings of the groups fortified with drinking water, and by injection by vesicle and control group at the level of $p < 0.05$ and $p < 0.01$, as calculated percentage of depreciation by (X^2) were used.[23]

RESULTS AND DISCUSSION

Clinical Signs

Whether vaccinated by drinking water or dose inside the crop. After the challenge, the vaccination of the two methods mentioned above showed simple clinical signs, starting from the fourth day of the challenge until the tenth day, marked by lethargy and slight wings of the wings with mild diarrhea by small amounts of blood in Guano sometimes. While the control group chicks (non-vaccinated) showed severe clinical signs and clear starting from the fourth day of the challenge represented by inactivity and lack of feed consumption due to loss of appetite, feathering, as well as roughness and wings dropping with pallor combs and wattles, noting that the chicks gathered in one place to warm up and acute bloody diarrhea.

Calculation of the Percentage of Mortality

The non-vaccinated control group recorded non-significant differences ($p > 0.05$) compared to the vaccinated chicks that did not record any death after vaccinated. In contrast, the significant differences ($p < 0.05$) between the control group and vaccinated groups became after the challenge, where the death rate was 10% in the control group compared, the vaccinated groups did not record any death (Table 2).

The absence of significant differences in the percentage of dead chicken between vaccinated and non-vaccinated groups after the challenge confirms that vaccination has no negative effect in increasing the percentage of mortality. But, after the challenge, the cause of no deaths in vaccinated chicks was due to the vaccination. Vaccination played a big role in reducing the severity of the disease, which led to fewer deaths, as a result of the immunity generated by vaccinated chicks compared to control groups that recorded a high rate of mortality, as they were exposed to large numbers of Eimeria Oocysts for the first time in the challenge dose that caused a severe injury. Therefore, severe clinical signs appeared, as they did not take the vaccination dose, and that there were no deaths between vaccinated chicks after the challenge, its results were better than what the researchers reached[27] when using two groups of chicks, one of them was vaccinated with a live vaccine (Praecox). The second was given an anticoccidial vaccine; the first group after the challenge recorded a mortality rate of 3%, and the second recorded 3.8%. Whereas the percentage of deaths among vaccinated chicks after the challenge of the current study was 0%. The results were similar to what Hassan found [15] when using the live vaccine.

Table 2: Experiment to calculate the percentage of mortality

Experiments	*Chicken after vaccinated*	*Chicken after challenge*
Control group	%^{a}5	% ^{a}10
Vaccinated with water	0 ^{a}	0 ^{b}
Vaccinated in crop	0 ^{a}	0 ^{b}

• Small letters indicate the significant differences ($p < 0.05$) between the experiments vertically

Packed Cell Volume

Significant differences were observed at $p < 0.05$ in average percentages of packed red blood cell volume after seven days of vaccination in vaccinated groups with water which had a rate of 30.7% compared to vaccinated with crop and control groups that did not show significant differences, which had averaged percentage 32.2 and 32.7%, respectively. After the challenge, significant differences ($p < 0.05$) appeared, as the control group recorded a decrease in the average volume of packed blood cells, which amounted to 29.2% compared to Vaccinated with crop and water, which was 31.7% and 31.8%, respectively. Significant differences $p < 0.05$ between the same treatments after vaccination were compared to their values after the challenge. The control group recorded a rate after vaccination, which was 32.7%, compared to the rate after the challenge, which was 29.2%; vaccinated groups with water and in crop did not record any significant differences between their rates after vaccination their value after the challenge Table 3.

The absence of significant differences in the average percentage of packed red blood cell volume between vaccinated and non-vaccinated chicks after vaccination confirms that the vaccine did not cause intestinal bleeding. After the challenge, the average percentage of packed red blood cell volume decreased significantly in the control groups, confirming that

Table 3: Packed cell volume (PCV)%

Experiments	*Chicken after vaccinated (%)*	*Chicken after challenge (%)*
Control group	32.7^{aA} ± 0.67	29.2^{bB} ± 0.31
Vaccinated with water	30.7^{bA} ± 0.67	31.7^{aA} ± 0.42
Vaccinated in crop	32.2^{aA} ± 0.98	31.8^{aA} ± 0.31

• Small letters indicate the significant differences ($p < 0.05$) between the experiments vertically

• Large letters indicate the significant differences ($p < 0.05$) between the experiments horizontally

the control group's chicks were affected by the large number of Oocysts after the challenge, and they caused bleeding in the intestine and cecum, which led to a lower percentage. While the effect of vaccination was evident in reducing the severity of macroscopic lesions, the lack of blood lost with chicken stools led to a high rate. This is consistent with what he found[28] and [29] in that the average percentage of packed red blood cell volume decreases at a high rate in cases of severe injuries.

Weight Increasing Rate (g)

No significant differences were recorded at the $p > 0.05$ level when measuring the rate of weight increasing between vaccinated and non-vaccinated groups after vaccination. After the challenge, significant differences appeared at the level $p < 0.05$ between the experiments. The average weight increased in the control group, it was 2170 g, followed by the vaccinated group with water, 2054 g, and the lowest vaccinated in the crop, which recorded 1866 g (Table 4).

The absence of significant differences in the rate of weight increased between vaccinated and non-vaccinated chicks after vaccination confirms that vaccination did not negatively affect the weight increase. But after the challenge, the absence of significant differences in the rate of weight gain between the control group and the vaccination group with drinking water compared to the weight gain that the vaccination groups recorded by the crop confirm that the vaccination by drinking water did not negatively affect the weight gain.[30] It differs from the outcomes[8] that the vaccine negatively affects weight gain for vaccinated chicks. Because of the number of vaccinated chicks with living Eimeria oocysts in the suspended solution prepared (this vaccine) used in this experiment, they were less than in the vaccine that they used the above research, therefore did not affect feed consumption and therefore did not affect weight gain.

The intensity of Severity Macroscopic Lesions in the intestine and the Cecum

The results showed that there were no macroscopic lesions in the intestine and cecum after seven days of inoculation in all tests. However, after the challenge, the results indicate the presence of significant differences $p < 0.05$ in the severity of macroscopic lesions of the intestine and cecum between vaccinated and non-vaccinated groups after seven days of challenge (Figures 2 and 3), where the control group recorded 2.3 and 2.8, respectively, and these conceders higher than in the vaccinated groups with water which ranged from 0.7 and 1.3, respectively, while the rates of the supplemented group in the crops were 0.6 and 1.7, respectively. There were no significant differences between the macroscopic lesions' severities in the vaccinated chicks according to the different vaccination methods (Table 5).

The reason that there are no macroscopic lesions in the intestine and the cecum of the vaccinated group after vaccination explains that the small numbers of Eimeria oocysts do not cause visible macroscopic lesions, and this is consistent with the current study[31] when used 40 Eimeria oocysts in the vaccinated chicken of the type Habbard orally injected daily from 1 to 14 days old, no macroscopic lesions

Table 4: Weight increasing rate (g)

Experiments	*Chicken between 25–32 days*	*Chicken between 25–48 days*
Control group	641[a] ± 62.1	2170[a] ± 75.2
Vaccinated with water	600[a] ± 24.4	2054[a] ± 82.3
Vaccinated in crop	596[a] ± 31. 9	1866[b] ± 119.6

Small letters indicate the significant differences ($p < 0.05$) between the experiments vertically
± Standard error

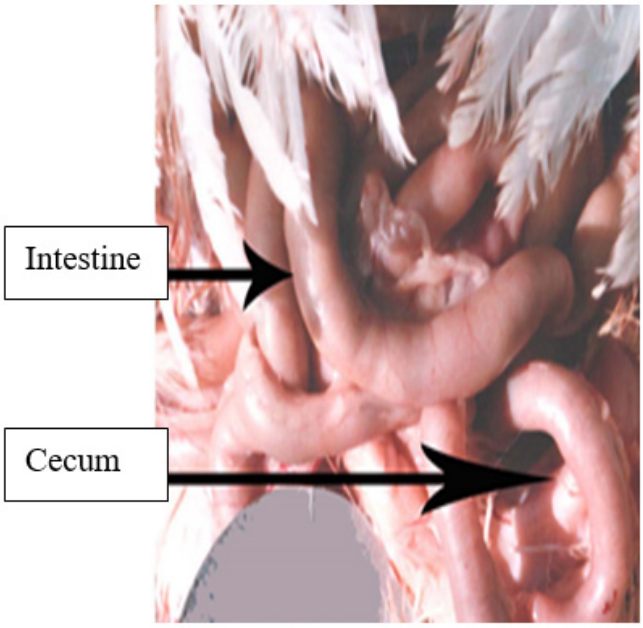

Figure. 2: The average of severity of macroscopic lesions in the intestine and the cecum after challenge

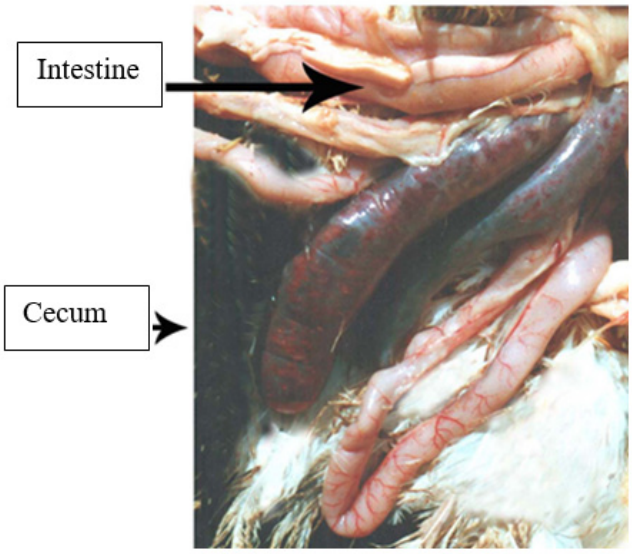

Figure 3: The average of severity of macroscopic lesions in the intestine and the cecum of dead chicken in control group after challenge

Table 5: The intensity of severity of macroscopic lesions in the intestine and the cecum

	Chicken			
	cecum		*intestine*	
Experiments	*After challenge*	*After vaccinated*	*After challenge*	*After vaccinated*
Control group	2.8^{a} ± 0.17	0	2.3^{a} ± 0.21	0
Vaccinated with water	1.3^{b} ± 0.21	0	0.7^{b} ± 0.21	0
Vaccinated in crop	1.7^{b} ± 0.31	0	0.6^{b} ± 0.21	0

- Small letters indicate the significant differences (P <0.05) between the experiments vertically
- ± Standard error

Table 6: The average number of Eimeria oocysts in Guano (gm/guano)

Experiments	*Chicken after vaccinated*	*Chicken after challenge*
Control group	0	185350^{a} ± 55304
Vaccinated with water	0	17833^{b} ± 1415
Vaccinated in crop	0	20217^{b} ± 2485

- Small letters indicate the significant differences (P < 0.05) between the experiments vertically
- ± Standard error

Table 7: The percentage of protection

Vaccination type used for chicks	*the percentage of protection in chicks (%)*
Using water	90.378
Using the crop	89.092

were observed after vaccination. And that the presence of severe macroscopic lesions in the intestine and cecum of control groups after the challenge compared to the macroscopic lesions in the vaccinated groups, whose intensity appeared much less, which indicates that the vaccine played an effective role in reducing the intensity of the macroscopic lesions of the protected chicks[31] and the results of the current study were close to what the researcher Hassan found.[15] The difference in the intensity of macroscopic lesions in the intestine from that of the cecum after the challenge is due to the difference in the numbers of oocysts and their types in the vaccine used, and this is consistent with what the researchers found.

The average number of Eimeria **oocysts in Guano**

The results indicated that there were no Eimeria Oocysts in the feces of the chickens after seven days of vaccination. After the challenge, Table 6 shows the presence of significant differences at the level of p <0.05 in vaccinated and unvaccinated chickens, The highest mean numbers of Eimeria oocysts in the control group were 185,350 Oocysts/gram of Guano compared to the groups vaccinated with drinking water and crop which reached 17833 and 20217 oocysts/g of Guano respectively. No significant differences were observed between the numbers of oocysts presented with vaccinated chickens, whether vaccinated using drinking water or in the crop.

The absence of Eimeria oocysts in Guano of vaccinated chicks after vaccination confirms that the vaccine was effective in inducing an immune response and, therefore, did not cause lesions due to obstructing the parasite reproduction, which led to reducing the numbers of Eimeria oocysts in Guano to zero. The presence of large numbers of Eimeria oocysts in Guano in control groups compared to the few numbers presented by vaccinated groups in both methods after the challenge confirms that there is an immune response generated by the vaccinated group, which led to obstruction of the parasite reproduction and thus reducing the number of Eimeria oocysts in Guano, and the preparation of Eimeria oocysts in Guano is considered one of the strongest indicators that many researchers rely on to evaluate the criteria used to control diabetes mellitus, as stated in the studies[12,31] The absence of significant differences between the numbers of oocysts presented with the Guano according to the vaccination method confirms that the two methods' efficiency is close.

Calculation of the Percentage of Protection

When calculating the percentage of protection for vaccinated chickens based on the calculation of the number of Eimeria oocysts in Guano after examining the challenge result shown in Table 7.

The absence of clinical signs in vaccinated chicks after vaccination confirms that vaccination did not negatively affect the health of the chicks. So the Eimeria Oocysts number in the prepared suspended solution used in vaccination didn't allow the development of the disease and compatible with the results of many researchers[25] after vaccinating the chicks using live Eimeria oocysts, and that the emergence of severe clinical signs in the control group in unvaccinated chicks after the challenge compared to the mild clinical signs in vaccinated chicks groups. And it was confirmed that vaccination has an impact on protecting chicks from this disease, which reduced the clinical signs in vaccinated chicks. This agreed with what the researchers found[26] when using live Eimeria oocysts as a vaccine. The protection ratio by using a suspended solution prepared with 50 *E. tenella*Oocysts and the other percentages for other types of living Eimeria oocysts. A percentage of protection of 90.378 was recorded in vaccinated chicks with drinking water after 7 days of vaccination, while 89.092 was recorded in vaccinated chicks in a crop after 7 days of vaccination, and this protection is close to what was recorded by Al-Adhami[33] and Al-Araji[34] when using oocysts attenuated by irradiation. In the vaccination, and better than recorded by both[35] and [36] who used the non-living materials of the parasite in the vaccination of chicken meat chicks, similar to what was recorded by Hassan.[15]

CONCLUSIONS

1. The use of the prepared local vaccine containing 50 *E. tenella* oocysts along with the other proportions of other types of Eimeria gave high immunity for chicks.

2. The use of mixed vaccine prepared with seven types of live Eimeria chicken has contributed to immunization of chicken of these types by reducing the proportion of mortality and clinical signs of the disease and reducing the severity of macroscopic lesions of the intestine and cecum significantly.
3. The prepared vaccine showed an increase in the percentage of packed red blood cell volume in the vaccinated groups after the challenge. This is evidence that the vaccine reduced the amount of bleeding from the intestine compared to the control group that lost large amounts of blood, which led to the reduction of the percentage of packed red blood cell volume.
4. The prepared vaccine did not negatively affect the rate of weight gain in the group that was fortified by drinking water.
5. Eimeria Oocysts were also reduced in Guano for inhibited chicks compared to high numbers in control groups, indicating the prepared vaccine's effectiveness to provide a high level of protection in chicks.

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