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Morphology and Life History of *Ascaridia galli* in the Domestic Fowl that are Raised in Jeddah

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ABSTRACT. During the survey of helminth parasites of domestic fowl in Jeddah, adult worms of *Ascaridia galli* were collected and studied from their natural infection. The life cycle of this nematode had been experimentally worked out for the first time in Saudi Arabia. Fertilised eggs when incubated at 28-30°C, developed to the infective stage in about three weeks. During this time, the first moult had occurred.

Completion of the life history of *A. galli* in Ross Broiler chickens fed with infective eggs and examined at 5-day intervals for 60 days showed that the other three moults of this nematode were observed on the 10*th*, 15*th*, and from 20*th* to 25*th* days after inoculation. Prepatent period was 37 days.

The morphology of mature male and female *Ascaridia galli* as well as the different stages of embryonic development and larval stages of this species are presented. This appears to be the first report of this nematode in Saudi Arabia.

Introduction

Although the name *Ascaris galli* was given by (Schrank) Freeborn[1], yet he did not describe the parasite, but based the species on a composite species, *Ascaris teres*, a nematode that had been found in dogs, cats, chickens and raptors.

Cram[2] regarded *A. perspicillum* to be a synonym of *A. galli*.

Bhalerao[3] listed four species, *A. lineata, A. galli, A. granulosa* and *A. compar* as parasites of domestic fowl in India. Baylis[4], however, considered two of them, *viz.*, *A. lineata* and *A. granulosa*, as the synonyms of *A. galli*.

Deol[5] described three species, *A. galli, A. columbae* and *A. compar* from Indian domestic poultry.

Nath[6] stated that a closer scrutiny of characters on which *A. perpicillum, A. lineata, A. granulosa, A. hamia* are differentiated from *A. galli* revealed that the validity of these species was unsound and, therefore, were considered identical to *A. galli*. Mozgovoy[7] stated that the definitive hosts of the order Galliformes have been reported to harbour 21 members of the genus *Ascaridia.*

From the foregoing records, it can be concluded that *Ascaridia galli* is by far the most commonly encountered nematode and cosmopolitan in distribution. The incidence of infection of this parasite was estimated by many authors in various parts of the world. Ackert[8] in Canada, estimated the incidence of infection of *A. galli* in fowls as 49%. In Tennessee, U.S.A., Todd[9] reported an infection rate of 42.5% among fowls. Reid[10] estimated the incidence in Egyptian fowls as 27.5%. Chand[11] found that 60% of the examined fowls in India harboured *A. galli.* In Russia, Kornishina[12] stated that the rate of *A. galli* infection among fowls reached 65.5%, while in Poland it was found by Dzido[13] to be 28%. *A. galli* was also found among other ten species of nematodes, six species of cestodes and three species of trematodes in *Gallus domesticus* from Thailand[14]. All chicken had nematode infections, while the infection rate of cestodes and trematodes was 93.3% and 13.3%, respectively. In Pakistan, Pal and Ahmed[15] examined 1568 guts of domestic fowls for helminth parasites and found that the specific infection for *A. galli* was 26.70%. During studying the incidence of helminths of fowls reared on deep litter and cage system in Bangalore, Hemalatha *et al.*[16] revealed the occurrence of *A. galli* in both systems of management. The rate of infection was higher in deep litter system that in the cage system.

Chickens and their intestinal roundworm, *Ascaridia galli,* have been utilized in laboratory experiments in parasitology for more than 50 years. Ackert *et al.*[17] described an easy method for obtaining and culturing *Ascaridia galli* eggs. Riedel[18], while using this procedure found that many cultures were destroyed by invasion of mycelia. In another study, Riedel[19] developed an improved technique in which the eggs were left in the uteri during the period of incubation. Hansen *et al.*[20] devised another method for culturing ascarid eggs. In that technique, an artificial digestive juice was used to free rapidly ascarid eggs from the uteri of female worms. According to Moran and Mizelle[21] and Nath[6], the first moult of *Ascaridia galli* occurs in the egg before the hatching of the larva.

Concerning the habitat and the life cycle of *A. galli,* Ackert[22] found that larvae of the fowl nematode *Ascaridia galli* burry their anterior ends deeply between the intestinal villi and into the glands of Brunner, but that they seldom pass through the wall of the intestine or migrate over the body of the host. Similar findings were reported by Roberts[23]. Ackert[24] studied the life history of *Ascaridia lineata* and recorded the time, the fertilised eggs and young worms require to develop to embryonation and maturation, respectively. The same author indicated that the habitat of *A. lineata* is the duodenum, especially the portion which is a few centimeters posterior to the entrance of the bile duct.

Moran and Mizelle[21] revealed that infective eggs of *Ascaridia galli* hatched in the anterior third of their host's intestine as early as 30 minutes after inoculation and most eggs hatch during 24 hours. Hansen *et al.*[25] proved that the intact shell protects the larva as it passes through the oesophagus, crop, proventriculus and gizzard. The early development of *A. galli* in its host has also been reexamined by Kumar and Tewari[26]. These authors reported that the parasite could enter the intestinal lining but was also capable of developing in the lumen without migration to the tissues.

Material and Methods

A total number of 545 chickens bought from different farms and markets in Jeddah were dissected and examined for any intestinal nematodes. A considerable number of adult *Ascaridia galli* were collected from the viscera of the infected birds. The collected specimens were fixed in hot glycerine alcohol, cleared in lactophenol and examined for identification.

For embryonic development, fertilised eggs were teased out from the uteri of the gravid female worms and incubated in distilled water at 28-30°C for 21 days. Two drops of 5% formalin were added to the culture medium to prevent bacterial and fungal growth. Eggs were examined daily and the different embryonic stages were recorded throughout the observation period.

For completion of the life cycle, two weeks old Ross Broiler chickens were fed, each with 100 embryonated *Ascarid eggs.*

Five days after inoculation of the eggs and continuing at 5-day intervals for the following 30 days, some chickens were killed, dissected and examined for the developing worms. Other chickens were kept alive till the end of the experiment (60 days) and their feaces were examined daily for *Ascaridia gallil* eggs to determine the prepatent period, duration and peak of oviposition.

Concentration floatation technique was used in the collection of eggs from feacal samples and eggs were counted with the McMaster slide. For the collection of the lumen larvae, the small intestine was quickly removed from the dissected birds and divided into six sections, of approximately equal size. The intestinal content of each portion was flushed into a glass jar, with hot water under pressure. Different developmental stages of this nematode were collected throughout the time of experiment, preserved in glycerine alcohol and cleared for study in lacto-phenol.

Results

I. Morphology of Ascaridia galli (Schrank, 1788) Freeborn, 1923

Parasitological examination revealed that 165 (30.3%) of the examined fowls were harbouring *Ascaridia galli.* Adult worms are yellowish white in colour and semitransparent. Cuticle is distinctly striated and the cuticular alae are feebly developed. The oral opening is surrounded by three prominent trilobed lips. Two conspicuous papillae occur on the dorsal lip and one on each of the subventral lips. A pair of the so-called neck papillae occurs on the sides of the body near the anterior end (Fig. 1a).

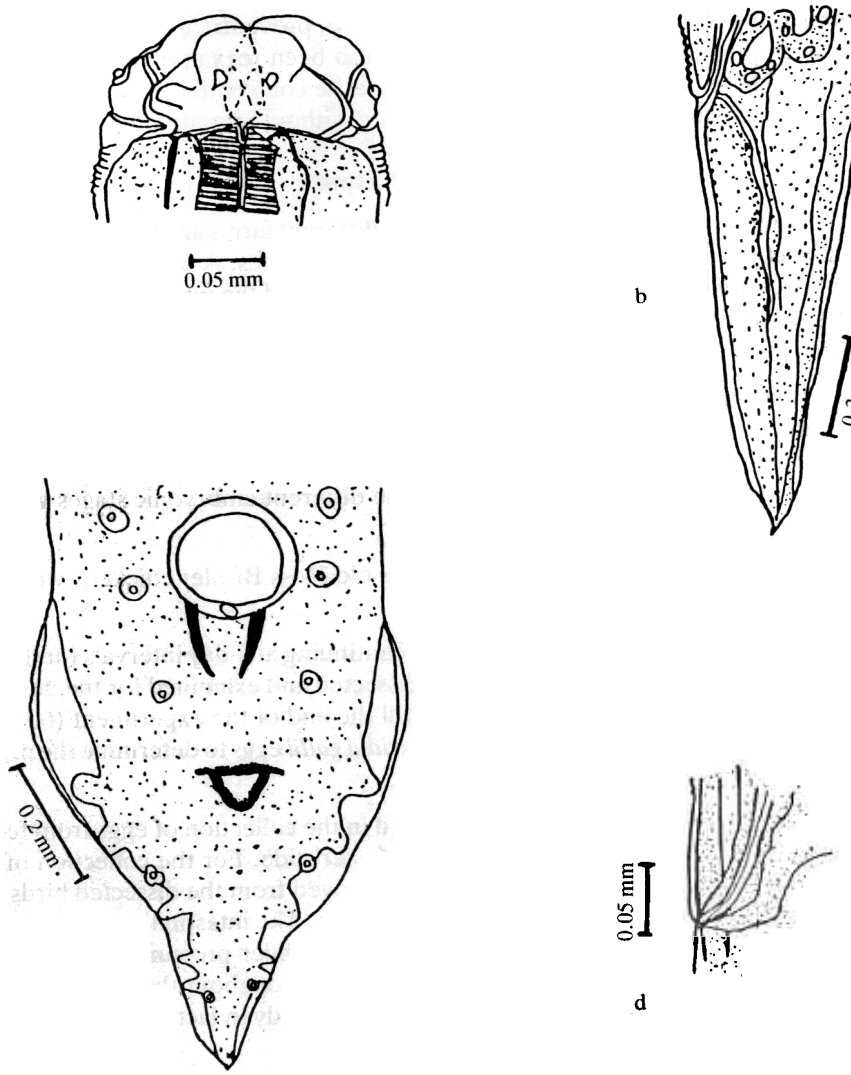


FIG. 1. *Ascaridia galli* (Schrank 1788) Freeborn 1923.
a. Head magnified to show lips and cephalic papillae.
b. Post. end of male to show caudal papillae.
c. Tail in female.
d. Vulvar region in female.

The Male

The male measures 42 to 76 mm (mean 63 mm) in length, and 0.56 to 0.91 mm (mean 0.77 mm) in maximum breadth. Oesophagus measures 2.48 to 5.32 mm (mean 3.85 mm) in length, and 0.28 to 0.59 mm (mean 0.47 mm) in breadth. Nerve ring lies at a distance of 0.48 to 0.92 mm (mean 0.68 mm) from the anterior end of the body. Excretory pore is 0.88 to 1.30 mm (mean 1.13 mm) behind the anterior end. Precloacal sucker varies in outline from oval to circular; longitudinal diameter 0.16 to 0.28 mm (mean 0.23 mm), transverse diameter 0.16 to 0.26 mm (mean 0.23 mm) and bears a minute ring at its posterior margin. Cloaca is a transverse slightly tongue-shaped slit in a distinct prominance, that lies at a distance of 0.27 to 0.40 mm (mean 0.35 mm) from the procloacal sucker. Tail is 0.57 to 0.78 mm (mean 0.70 mm) in length. Caudal papillae are ten pairs, lie on the ventral surface of the caudal end and arranged in distinct groups, *i.e.* precloacal (three pairs), cloacal (one pair); post-cloacal (three pairs)and subterminal (three pairs).

The well developed spicules are of about equal length and measure 1.2 to 2.9 mm (mean 2.4 mm); (Fig. 1c).

The Female

It is longer and stouter than the male and measures 72.0 to 108 mm (mean 85 mm) long. Its width at the vulvar region is 0.9 to 1.8 mm (mean 1.2 mm). Nerve ring lies at a distance of 0.44 to 0.84 mm (mean 0.75 mm) and excretory pore 0.7 to 1.5 mm (mean 1.1 mm) from the anterior end. Oesophagus is 2.88 to 4.24 mm (mean 3.2 mm) long, and 0.38 to 0.49 mm (mean 0.41 mm) broad. Vulva opens at a distance of 28.1 to 57.4 mm (mean 48.6 mm) from the anterior end. The anus is situated at 0.88 to 1.52 mm (mean 1.35 mm) from the posterior end of the body. (Fig. 1b, d).

II. Embryonation of A. galli Eggs (Fig. 2: a-q)

Most of the fertilised eggs divides into the two-cell stage within 24 hours of incubation.

The second segmentation took place in the next day giving rise to the three-cell stage. The four-cell stage was completed within three days in most of the eggs. In about 3 days, cleavage progresses to the morula with large blastomeres, after which more divisions occur simultaneously and the morula with small blastomeres is reached in about five days. As development proceeds the cells become smaller, and those at one end of the embryo were less obaque. Between the more and less granular cells, a line of separation appears, and, in about three days, the "tad pole" stage develops. The latter is with blunt anterior end and conical posterior tail. Two additional days of incubation result in the development of a vermiform embryo. Within the next three days, it transformed into the infective larva by continued increase in length and decrease in width, and by further reduced terminal opacity. Only one ecdysis appeared to have occurred during this development.

III. Morphology of the Different Larval Stages of Ascaridia galli

Chickens that were killed 5 days after being experimentally infected with em-

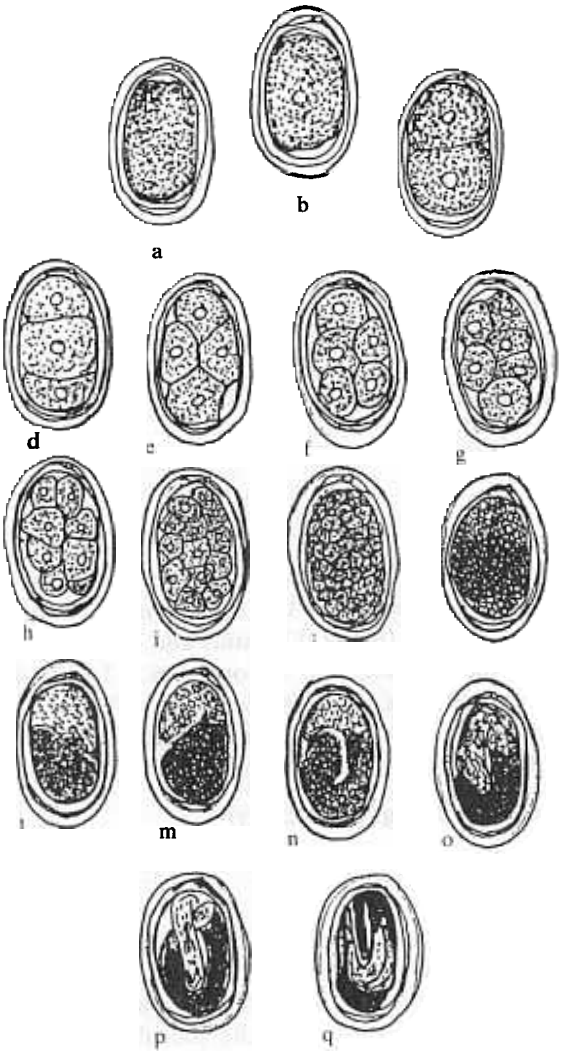


FIG. 2. Embryonation of *Ascaridia galli* eggs (a - q).
a – Mature infertile egg.
b – Fertile egg.
c-h – Eggs in cleavage stages.
i – Morula with large blastomeres.
j – Morula with small blastomeres.
k – Initial stage of differentiation.
l-m – "Tad pole" stages.
n,o – Early veriform embryos.
p.q – Coiled embryos (embryonated eggs).

bryonated *Ascaridia* eggs, showed the newly hatched larvae (Fig. 3) in the lumen of their intestines. The collected larvae measured from 0.3 to 1.0 mm (mean 0.8 mm) long, and were not well differentiated. The intestinal canal was filled with coarse granules. The oral opening was simple pore devoiding distinguishable lips and the tail tapered posteriorly.

Chickens killed on the 10*th* day harboured in their intestines lumen larvae after the second moult had taken place, including the presence of the nerve ring and the characteristic changes in the tail. The male which was 10 days old showed the typical sickled-shaped tail and the beginning of the preanal swelling (Fig. 4) which indicates the position of the future preanal sucker. Anal prominence although present, was not distinct. The corresponding female had a simple long tail (Fig. 5).

Measurements of these larvae give the following proportions: The larva to develop into a male measures from 2.6 to 3.08 mm in length (mean 2.3 mm). Oesophagus is 0.34 to 0.35 mm long. The nerve ring and the excretory pore lie at a distance of 0.16 to 0.18 mm, and 0.18 to 0.2 mm, respectively, from the anterior end. The sickle-shaped tail is 0.085 to 0.10 mm long.

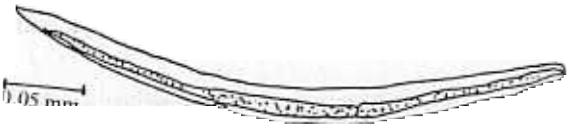
The female larva measures 3.2 to 3.6 mm in length. Oesophagus is 0.33 to 0.35 mm long. The nerve ring and the excretory pore lie at a distance of 0.18 to 0.19 mm, and 0.21 to 0.22 mm, respectively, from the anterior end. The tail is 0.13 to 0.17 mm long.

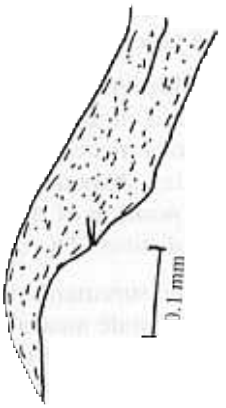
At the 15*th* day, it was observed that the third moult had occurred in different larvae. The principal changes common to both sexes were: mouth with distinct lips (Fig. 6); male with prominent anus and preanal sucker, and with only three pairs of caudal papillae (Fig. 7); in female, presence of vulva and short vagina with a proportionately shorter tail (Fig. 8).

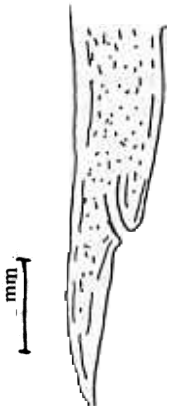
Such male larva is 4.8 to 5.6 mm (mean 5.2 mm) long by 0.09 mm wide. Oesophagus measures from 0.35 to 0.36 mm in length. The nerve ring and excretory pore are situated at 0.198 to 0.203 mm and 0.235 to 0.257 mm distance, respectively, from the anterior end. The tail measures 0.173 to 0.185 mm in length.

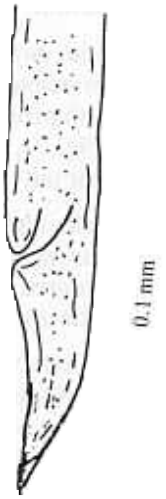
The female larva is 5.8 to 6.4 mm (mean 6.2 mm) long; with a maximum width of 0.10 mm. Oesophagus is 0.36 to 0.37 mm long. The nerve ring and the excretory pore lie at a distance of 0.207 to 0.218 mm and 0.244 to 0.273 mm, respectively, from the anterior end, and the vulva is located at a distance of 2.42 to 2.64 mm from the anterior end. The tail is 0.187 to 0.198 mm long.

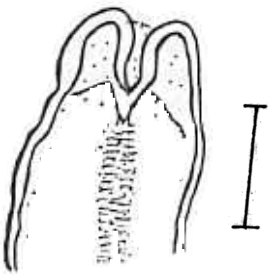
The 20-day larvae showed marked variations in development. The larvae had grown in size and oral lips showed distinct papillae; Fig. 9. Evidence of the fourth moult occurred daily from now until the 25*th* day when a specimen was found with the old cuticle distinctly separated from the new. Differentiation between the male and female larvae was possible from the study of the posterior ends which in males were provided with the preanal sucker, papillae and weak spicules; Fig. 10. The vulvar region of the female larvae was more conspicuous; Fig. 11. Males on the 20*th* day averaged 14.6 mm × 0.22 mm and females 15.4 mm × 0.26 mm with simple pointed tail (Fig. 12).











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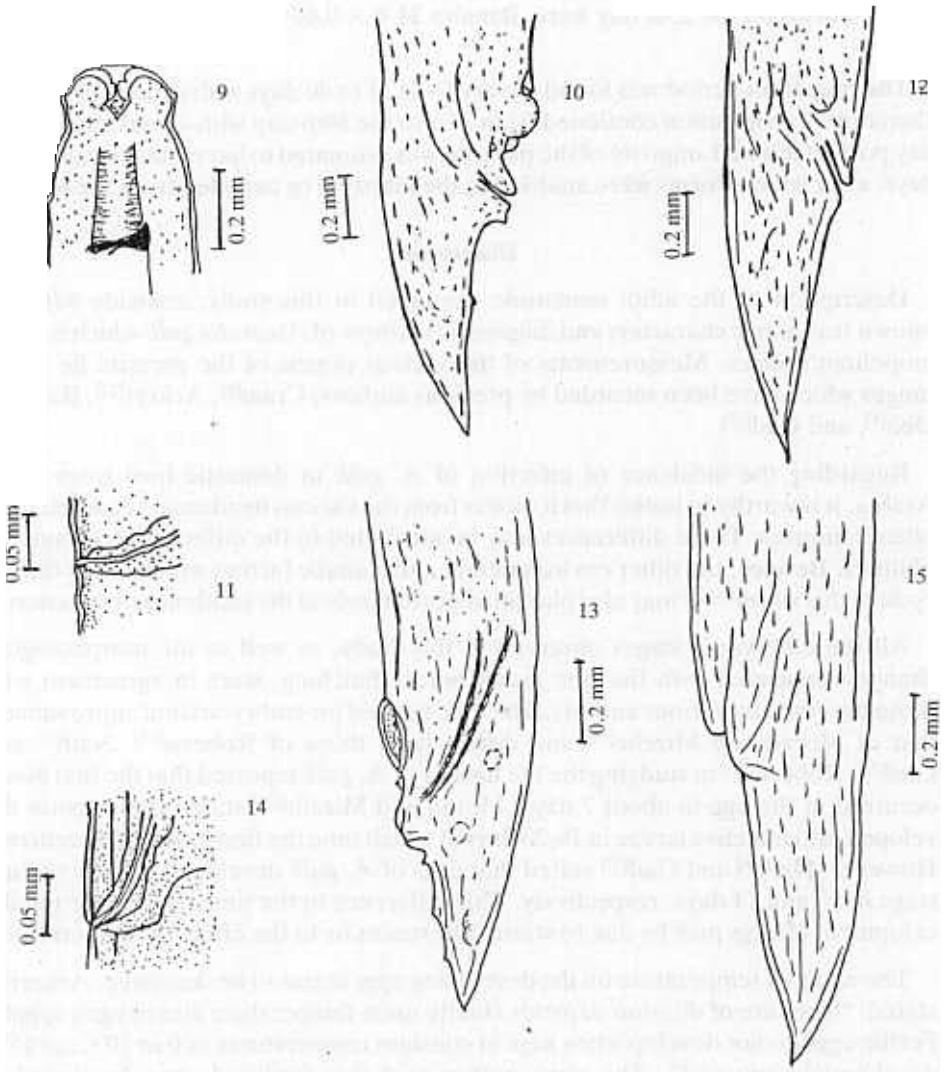

FIG. 9-12. Ant. end, post. end of ♂, vulvar region and post. end of ♀ 20 days-old larva;
FIG. 13-15. Post. end of ♂, vulvar region and post. end of ♀ 25 days-old (young) *Ascaridia galli.*

Chickens killed on the 25*th* day of infection harboured young *Ascaridia galli, i.e.* the fourth moult had taken place. In external characters the immature adult or the fifth stage larvae resembled the adults which were described from naturally infected fowls. In male, the posterior end carried a well-developed preanal sucker and ten pairs of caudal papillae. Spicules were well developed but were not fully chitinised; Fig. 13. The female showed slightly protruding lips of the vulva; Fig. 14. The vulva, vagina and uteri were well formed, but the uteri were without mature eggs and the tail was relatively longer and pointed (Fig. 15). Average measurements of a few

young worms on the 25*th* day were: females 24.6 × 0.68 mm and males 23.1 × 0.53 mm.

The prepatent period was found to vary from 31 to 40 days with a mean of 37 days. Duration of oviposition continued from 31*st* to the 60*th* day with a peak on the 45*th* day post infection. Longivity of the parasite was estimated to have a mean value of 60 days, after which worms were analised in the intestine or expelled from the host.

Discussion

Description of the adult nematode examined in this study, coincide with the known taxonomic characters and diagnostic features of *Ascaridia galli* which is a cosmopolitan species. Measurements of the various organs of the parasite lie in the ranges which have been recorded by previous authors; Cram[2], Ackert[22], Baylis[4], Deo[5], and Gad[27].

Regarding the incidence of infection of *A. galli* in domestic fowl from Saudi Arabia, it is worthy to notice that it differs from the various incidences recorded from other countries. These differences may be attributed to the different breed susceptibilities. Besides, the other environmental and climatic factors which affect the life cycle of this worm[10,27] may also play an important role in the incidence of infection.

All the embryonic stages observed in this study, as well as the morphological changes associated with the first moult before hatching, were in agreement with those observed by various authors. The time needed for embryonation approximates that of Moran and Mizelle[21] and differs from those of Roberts[23], Nath[6] and Gad[27]. Roberts[23] in studying the life history of *A. galli* reported that the first moult occurred in the egg in about 7 days. Moran and Mizelle[21] stated that zygotes developed into infective larvae in 16-20 days at which time the first moult had occurred. However, Nath[6] and Gad[27] stated that eggs of *A. galli* developed to fully mature stage in 13 and 14 days, respectively. This difference in the time needed for the development of eggs may be due to strain differences or to the effect of temperature.

The effect of temperature on the developing eggs seems to be decessive. Ackert[24] stated: "The rate of division depends chiefly upon temperature and oxygen supply. Fertile eggs do not develop when kept at constant temperatures of 0 or 10°C; at 15°C development proceed". The same author said that fertilised eggs developed to maturity in 16 days when incubated at 30°C; at 33°C they become infected in 10 days. Reid[28] stated that eggs of *A. galli* ceased full normal development when incubated at high temperature above 35°C or below 19°C. Gad[27] reported that preliminary refrigeration of *A. scarid* eggs for one day, followed by incubation at 28° produced a remarked change in the maturation period. The author indicated that preliminary freezing of the eggs delayed their development and killed a considerable number of them.

Also the morphological changes associated with the three moults of the parasite in the Ross Broiler chicken during the experiment were also recorded by Ackert[24] and Nath[6], while studying the life cycle of *A. galli*. The second author did not mention

the time needed for each moult, while Ackert[24] reported that the second moult of the lumen-larvae took place in the 6-8 day period, the third during the 14*th* and 15*th* days, and the fourth moult occurred in the 18 to 22-day period. It is evident that there was slight differences between these periods and those recorded in the present study. It can be hypothesized that the age of the host might have an influence on the development of the worms. Herrick[29] demonstrated that age was a factor in determining the rate of growth of individual worms of *A. lineata*. The same author found that the growth and chance of survival of *Ascaridia* was inversely proportionate to the age of the host.

Ackert[24] stated that *A. lineata* of fowls grew to maturity in 50 days in chickens parasitised when about a month old. Roberts[23] found fertile eggs of *A. galli* in the intestine after 27 to 28 days from time of infection with 100 *A. galli* eggs. Kerr[30] established the minimum measuring time of the prepatent period for *Ascaridia* in birds of different ages. In chickens infected when 12 days old, eggs were recovered from their faeces after 30 days of infection.

In this study, fertile eggs of *A. galli* were recovered by faecal examination in a minimum time of 31 days. This result is in good accordance with that of Kerr[30] and approximates that of Roberts[23].

None of the previous authors calculated the peak time where fertile eggs of *A. galli* were laid down and which was estimated 45 days in this study. Longivity of the parasite was also observed to have a mean of 60 days. After that time, some of the adult worms were analised in the intestine or discharged with the faeces. This finding may be attributed to age resistance of the host as mentioned by Herrick[29]. This author indicated that the resistance of chickens to the fowl nematode *A. lineata* increased with age up to 103 days after which no further increase was found.

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دراسة مورفولوجيا ودورة حياة ديدان إسكاريديا جالي التي تصيب الدجاج المنزلي في جدة

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المستخلص . عند حصر الديدان الطفيلية ، التي تصيب الدجاج المنزلي ، في جدة من خلال الإصابة الطبيعية جُمعت ودرست ديدان أسكاريديا جالي . ولدراسة دورة حياة هذه الدودة معمليًا ، أُجرى تحضين لبويضاتها المخصبة تحت درجة ٢٨-٣٠°م . وقد تم الحصول على الطور المعدي للبويضة خلال ثلاثة أسابيع حدث فيها الانسلاخ الأول للدودة .

ولتكملة دراسة دورة الحياة تم تغذية مجموعة من صغار الدجاج Ross Broiler عند عمر أسبوعين بالطور المعدي للبويضات ، وكانت العينات المصابة تُفحص كل ٥ أيام لمدة ستين يومًا من الإصابة . وقد لوحظ حدوث ثلاثة انسلاخات أخرى في اليوم العاشر والخامس عشر ، وفي اليوم العشرين - الخامس والعشرين من الإصابة ، وبلغت فترة الحضانة سبعة وثلاثين يومًا .

وُصِفَ كلٌّ من الشكل الخارجي لذكور وإناث الديدان البالغة ، كذلك تطور المراحل الجنينية واليرقية المختلفة لهذا النوع من الديدان أسكاريديا جالي .

وتُعتبر هذه الدراسة الأولى من نوعها التي سُجل فيها وجود هذا النوع من الديدان في المملكة العربية السعودية .