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A Review: *Haemonchus contortus* Infection in Sheep: Anemia, Epidemiology, and Advances in Diagnosis

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Abstract

HAEMONCHOSIS, global problem of sheep, is a major cause of death. The highly pathogenic parasite *Haemonchus contortus* lives in the abomasum of afflicted animals and causes disease by sucking blood, which has a negative impact on the animals' productivity and general health. It causes severe diseases and significantly reduces farmers' income. Thrives in warm, humid conditions, despite being historically more widespread in high-risk areas. Climate change and warming of newly discovered regions allow it to thrive. The infection is not evenly distributed throughout the world; it is more common in warm temperate, tropical, and subtropical regions with summer rainfall than in cool, cold, and arid regions. Diagnosis relies on clinical signs, followed by identifying genera and species to identify causative agents. This review explores anemia, The severity of the infection and the sheep's immunological response determine the possible outcomes, although in certain cases, an infection can be fatal. This paper aims to provide a concise overview of the information that has been and is currently available in the aforementioned areas in one location. An in-depth study was conducted to gather information on sheep haemonchosis and recommend sustainable control methods. The review used online databases and Semantic scholar to identify gaps and propose future research. Therefore, Different diagnostic techniques including morphological identification and microscopic examination, immunological and molecular methods also evaluated. In order to help readers completely comprehend the issue from a wider perspective.

Keywords: *Haemonchus cotortus*, Anaemia. Immunological diagnosis, molecular diagnosis.

Introduction

Haemonchus Contortus (*H. contortus*) is one of the most significant nematodes affecting sheep and goats in Egypt, causing haemonchosis, a severe disease that can lead to the death of lambs [1]. In many cases, chronic infections result in a marked decrease in productivity [2]. *Haemonchus Contortus* infections can manifest as either acute or chronic disease. Common signs of an acute infection include haemorrhagic anemia, dark stools, edema, weakness, reduced muscle mass, and sometimes sudden death. In contrast, chronic infections typically present with reduced appetite, weight loss, and anemia [3].

An accurate diagnosis is crucial when haemonchosis is suspected, as failure to provide timely and appropriate treatment can lead to significant and ongoing animal fatalities. It is equally important to rule out haemonchosis when it is not the likely cause, since some clinical signs, such as sudden deaths in livestock within endemic areas, are non-specific[4]. The identification of *Haemonchus* spp. can be achieved by using several methods, including fecal smears and fecal flotation to detect parasitic eggs, culturing eggs to obtain L3 larvae, and postmortem examination to identify immature and adult worms [5]. However, these techniques have low sensitivity and cannot differentiate between species or genotypes. In contrast, fecal culture methods for identifying nematode larvae, followed by PCR-based screening of positive samples, offer significant

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advantages and may provide a more accurate confirmation of infection[6].

This review examines anemia, epidemiology, and diagnostic methods for Haemonchosis, including morphological identification, microscopic examination, as well as immunological and molecular approaches abetter understanding of these factors wo uld lead to more effective diagnostic techniques, whi ch would lower the likelihood that the disease would manifest. In order to lessen the parasitosis's negative effects on livestock productivity and health, a number of components of the sheep
H. contortus interaction are examined in this paper

The severity of the infection and the sheep's immune response influence the potential outcomes, with some infections being fatal. The review provides a comprehensive discussion on the anemia associated with haemonchosis and the related clinical symptoms of hyperacute, acute, and chronic forms of the disease. Additionally, various diagnostic techniques are assessed.

Anaemia

In Egypt, *Haemonchus cotortus (H. cotortus*), one of the most important nematodes that infect sheep and goats., Its cause haemonchosis, is a serious disease led to death lambs [1] . In most cases, chronic disease results in a significant reduction in productivity [7]. *Haemonchus cotortus* infections can present either an acute or chronic disease. The most common clinical signs of an acute infection include haemorrhagic anemia, dark-colored stools, oedema, weakness, reduced muscle mass, and occasionally sudden death. In contrast, the most common clinical signs of a chronic disease are decreased food intake, weight loss, and anemia [8]. Anaemia in sheep can arise from a variety of diseases. Hemonchosis, in many countries across the world, is acknowledged as one of the major that causes of the anemia in small ruminants of all ages [9]. Thus, when examining cases of anemia or death in sheep, haemonchosis ought to be on the differential diagnosis list. FAMACHA (FAffa MAlan CHArt) is a widely used technique in tropical and sub-tropical nations that uses the color of the conjunctiva to visually assess anaemia. A score of 1–5 is used, with 1 and 2 (red or pink) denoting normal, 3 (light pink) denoting uncertainty, and 4 and 5 (pale) denoting anaemia [10]. Due to the life cycle of *H. cotortus*, which affects mucins, morphology, and gastric secretion; additionally, a Th2 biased host immune response is triggered, resulting in mast cell and eosinophil tissue infiltration [11]. A raised nodule surrounds the infected gland when infectious third-stage larvae (L3) emerge as L4 or immature adults after emerging from their rumen sheath and developing in abomasal glands [12]. The emergence of the parasite into the abomasal lumen is quickly followed by generalized effects on secretion, elevated abomasal pH, and serum concentrations of gastrin and pepsinogen. For *H. cotortus*, this usually happens after 2-4 days [12]. Hypoproteinemia occurs along with hemonchosis [13]. Numerous factors contribute to this hypoproteinemia, including as blood loss, *H. cotortus* haematophagous activity, broken cell junctions that allow protein to escape into the abomasal lumen, and enhanced permeability, reduced protein absorption because of tissue repair, abomasal epithelial cell loss, and elevated mucus production [14]. Additionally, there is a rise in the utilization of proteins to create an immune response and heal injured tissues [15] Reduced intravascular oncotic osmotic pressure and oedema, which can be localized or widespread in the intermandibular area, such as the bottle jaw or cervical region, are the outcomes of hypoalbuminemia [4]. The oncotic draw of plasma proteins, particularly albumin, aids in preserving blood vessel fluid. The fluid will move out of the vascular area if the plasma's albumin content is drastically reduced (typically less than 15 g/L)[16]. Since the typical daily losing of blood during infection of sheep is approximately 0.03 ml/parasite, the animal has already suffered damage . Although oedema can develop in any organ or tissue, it is more frequently observed in subcutaneous tissue and is more noticeable in areas with high hydrostatic pressures or in the lower body due to gravity. Although some sheep may pass away before the oedema develops, these circumstances may account for the development of oedema in the intermandibular region observed in acute haemonchosis. [4]. Compared to hyperacute haemonchosis and acute haemonchosis that is characterized by smaller loads of *H. cotortus* (about 2000–20,000 larvae per animal), which leads to a less severe blood loss and anemia that develops over as a longer period. Sheep experiencing acute haemonchosis exhibit symptoms such as weakness, lethargy, elevated heart and respiration rates, and pale mucous membranes, which are indicative of anemia and hypoproteinemia. Packed cell volume (PCV) decreases initially, but within the first 14 days of infection, compensatory erythropoiesis takes place, and full compensation and apparent recovery take place over the course of six weeks [16]. The primary tissue effects include the loss of parietal cells that secrete acid and morphological abnormalities in many of the parietal cells that are still present, though some are still viable and able to respond to stimuli [17]. Additionally, there are hyperplastic alterations, including larger pits with reduced mucin, a rise in the quantity of mucous neck cells (MNC), and zymogenic cells with an immature phenotype. When animals eat forage, they swallow the infectious stage of *H. contortus*, known as L3 larvae. Following feeding, several host sheep-derived variables affect the L3 larvae's survival in the udder. These include the health, age and status of nutrition of sheep, as these factors influence their capacity to develop an

immunological defense against *H. contortus* larvae [18].

Epidemiology

Although *H. cotortus* may thrive in a variety of climate zones, warm, humid conditions are ideal for its survival. Haemonchosis was historically more widespread in areas that were known to be high-risk; however, climate change and the warming of newly discovered regions of the earth seem to be allowing *H. cotortus* to live and thrive in areas that were previously low risk [19]. The free-living stages of *H. cotortus* require a warm, humid environment to survive and grow outside of the host [20]. In dry areas of the world, haemonchosis is uncommon because there isn't enough moisture for *H. cotortus* free-living larval stages. However, in warmer, arid regions, more rainfall or irrigation may help larvae survive . The ability of *H. cotortus* fourth larval stage to go through hypobiosis, or arrested development, is also significant because it helps the species survive in dry or frigid environments [21]. The ideal temperature is 26 ◦C and relative humidity (RH) of ≥ 80-90% for *H. contortus* egg hatching and larval development . Fernández-Ruvalcaba et al. [22] revealed the impact of seasons on the eggs development the infective larvae, and he made in the largest recovery of L1 and L2 in rainy season. The season that has highly rain thought to be an ideal time for *H. contortus* eggs to hatch and mature [23]. Moisture is one of the most critical climatic variables impacting the development of infective larvae.

It can take four days for an egg to develop into an infectious third stage larvae (L3) under the right environmental conditions, life cycle of *H. cotortus* was shown in Fig.1.

In any other case, the length of larval development varies more. Desiccation and low humidity quickly destroy eggs and larvae. The most resilient, free-living form, the ensheathed L3 larvae can live for extended periods of time in favorable temperature and humidity conditions. They also retain the cuticle of the second-stage larvae. After emerging from the excrement onto the soil, the L3 randomly move up the grass sword in both horizontal and vertical directions, regardless of the availability of free water [24] . The larvae survive by storing energy instead of feeding . Larvae can survive the winter in certain climates because they are inert, require little energy, and can endure temperatures below freezing for extended periods of time. Due to their increased activity and consumption of energy reserves, L3 larvae normally do not survive for longer than five weeks in tropical climates. This gives some geographical regions the chance to use spelling of pastures, or times without grazing, as a control measure . L3 larvae can be reached to the top peak numbers seasonally, depending on increased the moisture, because of higher rainfalls .Hypobiotic L4 larvae develop only during seasons which contain highly dry ,but in this situations have not been reported in such areas, because of L3 larvae were present nearly at all year-round [25]. The Infection's Changing Face the majority of epidemiological research on haemonchosis has been on the sequential observations of parasitic burdens in grazing ruminants,studies have shown the causal relationships between several environmental factors and the parasite's development.. Three methods have been used to base these epidemiological studies: (a) using animals as tracers, which grazed on pastures contaminated with parasite eggs and larvae at particular times; (b) counting the number of worms in faecal samples from ruminants that were naturally infected and constantly challenged by nematodes; and (c) conducting surveys at abattoirs. majority of grazing ruminants were infected by different species of nematodes, which is the main drawback of epidemiological studies that emphasize haemonchosis. Competition between these coexisting species affects the number of worms that are counted. There are a lot of epidemiological studies about *H. cotortus* in the literature, but not all of them define the disease's epidemiology over the course of a year. This failure is attributed to the notable variation in frequency and severity of observations between studies. However, they offer a chance to emphasize the impact of the season.Temperature and humidity affect nematode population ranges in different environments) to use appropriate antiparasitic methods. The previous studies evaluating fecal product counts should not be evaluated for epidemiological reasons, as they are not indicative of worm burdens. On the other hand, the main advantage of worm counts from livestock is the presence of larvae during hypobiosis, an additional way for *Haemonchus spp*. in adverse weather conditions [26]. A major weakness of epidemiological studies emphasizing genetic selection is that most ruminants are infected by different nematode species. These current species create competition -affecting the number of worms counted. Several epidemiological studies of *H. cotortus* are available in the literature, but not all epidemiology is reported in a single year, and this failure is attributed to the large number of differences between studies in the frequency and intensity of findings. However, they have the opportunity to show the effect of time (ie temperature-moisture effect) on the range of nematode populations between different environments so that appropriate antiparasitic strategies can be used [26]. The requirement for the free-living stages of *H. cotortus* governs is the warm and moist environment where the parasite's distributions. The prevalence of *H. cotortus* and disease in grazing animals is therefore particularly high in the equatorial climatic zones for both hemispheres, between latitudes 23.5°N and 23.5° S [27]. However, *H. cotortus* has proven and survive to

be remarkably adaptable to over wide range of environments , due nematodes have developed the ability to inhabit a broad array of ecological environments. While numerous species lead a free-living existence, parasitic nematodes depend on one or more hosts to fulfill their life cycle. Many of these parasitic forms experience a series of intricate morphological transformations, which are associated with their migration within hosts to establish a mature infection [27]. Their complex life cycles may include the involvement of intermediate hosts, vectors, and periods spent in the environment, where they encounter severe and fluctuating conditions such as frost or drought, which they must endure between host infections. Parasitic nematodes have shown remarkable adaptability to various threats, including predation, climatic changes, and the immune defenses of a diverse range of both plant and animal host species [28]. *H. cotortus* is a significant and aggressive blood-sucking parasite affecting small ruminants, primarily located in the abomasum. This parasite is responsible for substantial production losses globally, and its blood-feeding behavior leads to severe health issues, including anemia, diarrhea, weight loss, edema, recumbence, extreme weakness, and, in severe cases, death [29] . *H. cotortus* consumes approximately 0.05 mL of blood daily, either through direct ingestion or by exploiting lesions [30]. The condition known as haemonchosis is prevalent in areas where sheep and goats are farmed; however, the most significant economic impacts are observed in temperate and tropical climates. The disease has been identified in the cold climate and has recently can be detected as far north as the Arctic Circle [31]. A lot of risk factors contribute to incidence of haemonchosis, including characteristics of host and conditions of environment, as agro-ecological factors, husbandry practices, deworming schedules, and pasture management. Additional risk factors encompass host species, the sex of the animal, age, body condition, and race/genotype [32]. The global diversity of *H. cotortus* isolates was shown in figure 2. The species of parasites and the density of the worm population influence the progression of gastrointestinal parasitic infections [33]. This study examines the prevalence of *H. cotortus* in sheep reared in the Gharb plain, considering various risk factors such as the age and sex of the animals, the season, and their body condition. Occurrences of haemonchosis in arid and cooler climates are noteworthy. The broad climatic range may indicate that local strains have adapted to less favorable ecological settings. Furthermore, the observed rise in the frequency of outbreaks in regions not traditionally recognized as endemic for haemonchosis particularly in colder, temperate areas could be linked to changes in climate, summarizing epidemiological features of *H. cotortus* infections according to climatic changes were shown in table 1. While the risk of haemonchosis can differ significantly on a local scale, even in areas where *H. cotortus* is prevalent, comprehensive ecological studies offer a robust foundation for forecasting the relative geographical and seasonal risks associated with climatic factors [4].

Diagnosis

Morphological identification and microscopic examination

In the past, fecal investigations of suspected cases and an assessment of clinical symptoms have been necessary for the diagnosis of haemonchosis. Both strategies, though, have disadvantages. First, the clinical signs typically start to show themselves when the infection starts to heal. Furthermore, *H. contortus* has a brief prepatent period that lasts between 21 and 28 days, or roughly 4 weeks, following which the infection worsens, and eggs are found in feces. The identification of ruminant *Haemonchus* has relied on the use of conventional laboratory culture of eggs in faecal samples to the infective larval stage, and the identification of the larvae based on the dimensions and morphology of various structures, as the majority of these eggs cannot be reliably differentiated based only on size or morphology [34]. Clinical signs and microscopic analysis of stool samples are typically used to diagnose haemonchosis . The low sensitivity of the stool examination and unclear clinical signs lead to the correct diagnosis of the disease. This makes it more important than ever to correctly diagnose this harmful parasite in faces samples. Thus, accurate information regarding the species involved, particularly regarding the presence of *H. cotortus*, is crucial in routine gastrointestinal nematode testing. It is possible to identify *H. cotortus* in feces under a microscope by looking for eggs, or better yet, by using cultured larvae [35]. Up until now, a lot of ruminants, both domestic and wild, have had *H. cotortus* infections found using these diagnostic techniques. Up until now, a lot of ruminants, both domestic and wild, have had *H. cotortus* infections found using these diagnostic techniques. While microscopy is a reasonably inexpensive and dependable method, morphological identification of parasite stages in fecal samples necessitates technical staff with the necessary expertise. As a result, the approach is ultimately impractical in a routine diagnostic setting. The goal of the investigation influences the diagnostic approach selection as well. Establishing automated and ultrasensitive procedures that are more accurate, labor-saving, and time-efficient is urgently needed for some applications, such as the declaration of freedom from the parasite. Numerous examples from the literature show how gastrointestinal nematode eggs and/or larvae, particularly in sheep, can be identified to species level using various molecular diagnostic techniques[36]

Immunological methods for diagnosing haemonchosis

Anti-*H contortus* antibody detection in sheep serum using ELISA, a dependable serological assay that gets over the drawbacks of conventional techniques that depend on the use of certain immunogenic antigens[37]. When diagnosing haemonchosis serologically, a number of antigens made from the worm *H. contortus* are employed, each with varying effects on sensitivity and specificity [38]. According to the current study, the GST antigen may be a promising antigen for haemonchosis diagnosis [39]. According to the current ELISA results, when tested on sera from sheep that were naturally infected, all the various *H. cotortus*-prepared antigens had the highest sensitivities (90–100%). According to this, the ELISA assay is a trustworthy diagnostic method for identifying IgG from this kind of infection [40]. All antigens had high apparent prevalence scores (78.51–90.8%), except for the GST antigen, which showed a notable difference percentage (45.319%). However, statistically, there was a significant difference of less than 0.05 between the GST antigen and the other prepared antigen, indicating that GST was the superior one. This discovery may be linked to the purified antigen obtained through affinity chromatography, which has improved diagnosis outcomes [41]. With 70% sensitivity to identify anti-*H. contortus* antibodies as early as two weeks after infection, the GST antigen showed excellent specificity and diagnostic efficiency. It was also found to be useful in early antibody titer detection. [39]. False positive reactivity with an antigen may be due to immature worms, recent infection, previous antibodies, or anthelminthic drug-eradicated worms. Furthermore, even though it appears at 24.5 kDa on the gel, the immunogenic band at 25.5 kDa may oversee this antigen's species specificity [42]. This purified antigen's ability to stimulate IgG production against it may be the reason for the finding, which showed an intense binding reaction with blotting measurements of 25.5 kDa [43]. The presence of a 24.5 kDa reactive band on blotting may be the reason why ESA antigen can exhibit the same specificity as GST antigen. The presence of a common reactive band at 98.7 kDa that appears with excretory secretory antigen ESA and crude somatic antigen CSA on blotting may be the cause of the highest true positive number in infected examined sheep, even though ESA recorded lower percentages in all other parameters except apparent prevalence (82.34%), which was higher than GST antigen. This outcome might be sent back to ESA. Since antigen is a part of several antigenic macromolecules that are crucial for maintaining the immunological and physiological equilibrium between the parasite and its host, its components vary depending on the preparation techniques. Despite having the highest prevalence percentage (90.8%) and sensitivity (90%), the rhcp 26/23 antigen, a recombinant antigen of adult *H. cotortus*, demonstrated a notably high number of false positives in sheep sera that were not infected, which contributed to the low specificity (6%) of the test. The reason for this could be traced back to the protein's ability to successfully immunize sheep against haemonchosis [44], as opposed to its potential use in specific diagnosis and IgG detection against *H. cotortus*. However, the highest sensitivity (100%) was attained by both CSA and crude larval antigen (CLA) because of the highest true positive number in infected examined sheep sera, even though both tests also recorded high false positive numbers that resulted in 0 and 10% specificity, respectively. This could be linked to the shared immunogenic band at 58.7 kDa that shows up on blotting instead of the SDS-PAGE profile and the cross-reactivity with other helminthes [45]. This could be because the protein band was not widely distributed in the CSA and CLA antigen extract. Additionally, electroblotting was carried out in a way that allowed the band to transfer effectively, and the IgG antibodies of HIS-CSA and HIS-CLA were incubated for the entire night, resulting in the appearance of IgG-induced reactivity against this protein band. Thus, the GST purified antigen may be the most promising antigen to use as a biomarker for sheep haemonchosis diagnosis, according to the study's findings. According to the current study, the L antigen is the most skilled antigen for this type of serological diagnosis. Furthermore, the L and ESP antigens obtained the highest apparent prevalence values, at 92 and 75%, respectively.[39] for a more precise diagnosis, a trustworthy serological diagnostic assay is therefore required. In large-scale intensive sheep livestock breeding, ELISA facilitates both infection detection and sero-epidemiological investigations[46]. Traditional methods that rely on the use of specifically immunogenic antigens are limited when it comes to the detection of anti-*H. cotortus* antibodies in sheep sera using a reliable serological assay like ELISA[38]. The sensitivity and specificity for the numerous antigens made from the *H. cotortus* nematode used in for serological diagnosis of haemochosis [38]. In the order to characterize and evaluate the different prepared *H. contortus* antigens, L, ESP, and AS antigens in the serodiagnosis of sheep hemochosis, that was the target of our study. The current study indicated that the L antigen is the promising antigen for serological diagnosis of *H. contortus* infection in sheep because the immunodominant band at molecular weight 57 kDa is responsible for the specificity and accuracy of this antigen's positive predictive value. [47]. Nematode parasites are linked to two types of molecular entities: soluble excretory-secretory protein antigens and somatic antigens. Natural antigens trigger an immune response, while hidden antigens do not. Both types are internal or external[47]. It is necessary for target antigens

derived from the parasite to be accessible to antibodies and possibly other immune response components for them to be deemed a good target for diagnosis. Some parasitic species exhibit blood-feeding behaviors in which they release specific excretory-secretory molecules into the host's bloodstream. These molecules are closely linked to the parasites' gut surface[26] .

Diagnosis is frequently confused with identification or detection. Unfortunately, diagnosing illnesses and infections and identifying organisms are not always mutually exclusive activities. The diagnosis is typically based on a few clinical signs; identification of genera and species is then carried out to identify the causative agent. They involve the interaction of direct or indirect methods of detection. based on the structure and molecular characteristics for specimens, accurate and authoritative identification that is both time- and money-efficient lays on the groundwork for characterizing parasite diversity and shifting patterns of geographic distribution, host association, and disease emergence. Many methods that have been developed to be dated are predicated by strict host associations which exist between *H. placei* and between *H. cotortus* and small ruminants, such as sheep and goats. This assumption, it is shown by current research and mounting empirical evidence of natural infections in the field, misrepresents the host associations for these *H. species*. Other characteristics for studying the biology of hosts and parasites. ELISA and Western blotting are assays used for identification *H. specific* antibodies in the fluids of body, Like serum, saliva, stool and tears from live animals. There have been few notable technological advancements in literature, and these assays have been thoroughly described [48, 49]. Although ELISA makes it easy to perform these assays, which typically target serum IgG, it can be difficult to detect IgE and IgA in the bloodstream, which are crucial for comprehending diseases and are considered to be more significant than IgG when determining levels of host protection. This is because expensive reagents are not readily available. It is possible to accurately measure antigen-specific IgE and IgA in local mucosal tissues after a biopsy or postmortem [50] . As a result, these assays are very useful for laboratory research but less so for diagnosing hepatitis in living animals. With high sensitivity, accuracy, and reproducibility, capture/sandwich ELISA is typically used to reliably detect low levels of IgE and IgA in blood. Although it is commercially available, a broadly cross-reactive IgA sandwich ELISA that also detects ovine IgA does not appear to have been widely used in ovine studies. The measurement of this Ig subtype by ELISA has been made possible by the availability of commercially available antibodies against sheep IgE [51]. Using an antisheep IgE monoclonal antibody, 2F1, produced from a chimeric IgE protein, a sheep IgE capture ELISA was created [52]. Total IgE was found in intestinal homogenates and colostrum using an assay, but not in serum. Notably, resistant sheep infected with T. colubriformis showed higher levels of antigen-specific IgE than susceptible sheep [52]. One research method for determining the spatial location of isotype-specific antibody-containing cells in situ is immunohistochemistry. Tissue fixation, embedding, sectioning, rehydration, and probing with antiisotype antibodies labeled with a reporter enzyme are all steps in this process, which is followed by substrate detection. For example, [53] found that the abomasum of sheep infected with *H. cotortus* contained cells containing IgA, IgG1, IgG2, and IgM.The most prevalent cell types tested positive for IgA, IgG1, and IgM. The enzyme-linked immunospot assay has not been used to detect *H. cotortus* specific antibodies in small ruminants, but it was successfully applied to study *T. colubriformis* specific antibodies [54]. The frequencies of isotype-specific secreting cells in a mixed cell population in small ruminants infected with *H. cotortus* may be evaluated using this assay. Techniques for immunodiagnosis could be applied to detect antibodies or antigens in the infected serum. Accurate early disease diagnosis can be aided by carefully choosing the antigen to use. [38]

The identification of *H. cotortus* is particularly important when the estimated burden size determines whether treatment is necessary or not, and when narrow spectrum anthelmintics are specifically designed to treat helminths that feed on blood. Therefore, for a more accurate diagnosis, a reliable serological diagnostic method is needed. ELISA can detect disease and seroepidemiological studies in large-scale sheep farming [46]. Immune gastric mucosa is higher than normal tissues, its presence has been associated with protection in sheep infected with *H. cotortus* [55]. Live animals are limited because, despite being abundant in homogenates of abomasal tissue of parasite-immune sheep, it is low to undetectable in serum and lymph. Moreover, the antibody interactions may be disrupted by inhibitors found in sheep serum and lymph. Consequently, homogenates from abomasal tissue are ideal for use with ELISA. T cell proliferation assay T cells are a major component of peripheral blood mononuclear cells (PBMC) and have significant functions in both innate and adaptive immunity.. T lymphocytes. In addition to studying parasite molecules that can alter host immunity, antigen specific T cell assays can be helpful in assessing T cell responses to *H. cotortus* infection [56]. By binding to the surface of PBMC, including T cells [57], and specifically to the transmembrane protein 63A [58], parasite-derived galectins suppress immunity and thereby facilitate the infection process, according to research on isoforms of recombinant galectin from *H. cotortus*. T cell assays can therefore be very instructive. However, if T cell lines or clones are used, the identification and

characterization process is very time-consuming and involves three H-thymidine, homologous host cells as antigen presenting cells, and irradiated antigen presenting cells. Delineating the pathways involved in the infection process can therefore be influenced by describing the animal's immune state. For instance, adenosine and ADP levels were found to be significantly lower during *Haemonchus* infection, despite the fact that one might anticipate an increase in ATP levels due to cell damage [59]. adenosine and ADP play in regulating platelet activation and enabling blood feeding for *Haemonchus*. Additionally, this same assay revealed that sheep exposed to infection on pasture had systemic *H. cotortus* specific IgE, and that protection in lambs vaccinated against the *H. cotortus* antigen was more closely associated with IgE levels than with IgG1 levels [60]. More reliably and accurately detect key *H. cotortus* antigen specific IgA and IgE in ovine blood or other body fluids may make these antibodies useful for identifying population mucosal immunity and for the selection of animals that naturally resist *H. cotortus* infection due to high levels of IgA and IgE. LISPOT and the identification of antibody-secreting cells). Immunodiagnostic methods may be applied to detect antibodies or antigens in the contaminated serum. Accurate early disease diagnosis can be facilitated by careful antigen selection [47]. Sykes et al. [61] raised the prospect that various gastrointestinal infections could be identified in feces as opposed to serum. Primarily, antibodies specific to whole parasite crude extract were created and coated on microtiter plates. In a subsequent capture assay, coproantigen was detected and identified using the same or a different parasite-specific antibody. Since they can accurately and sensitively detect infections early in the prepatent period, immunodiagnostic assays for antigen detection are superior to others. Furthermore, because it avoids the issues of cross-reactivity and false negatives, coproantigen detection is still a promising method for use in large-scale labs or epidemiological studies. It may also be used to track how well treatments for these illnesses are working [39]. Even though various diagnostic methods for haemonchosis were described using various *H. cotortus* antigen types in enzyme linked immunosorbent assay (ELISA) [47]. there is still a lack of understanding regarding the characterization and application of coproantigen for early diagnosis. When diagnosing exposure or infection specific to *Haemonchus*, serological methods can be very informative. There are review papers on the immunological techniques that are used to identify haemonchosis by detecting IgG antibodies specifically [49]. Since antibody levels can persist long after the infection has cleared, these assays have generally not been widely used due to issues with antigen specificity and relevancy. Furthermore, the host typically shows clinical symptoms of infection long before the levels of *Haemonchus*-specific antibody titres rise to levels that can be consistently detected. Furthermore, there can be significant differences in serum antibody levels to infection between outbred animals. Cattle infected with *C. oncophora*, *Dictyocaulus viviparus*, and *Fasciola hepatica* can be distinguished using bead-based technologies for immunodiagnosing nematode infections [62]. However, like most immune-based essays, including those for haemonchosis, this test is genus-based rather than species-specific. Furthermore, there can be significant differences in serum antibody levels to infection between outbred animals. Cattle infected with *C. oncophora*, *Dictyocaulus viviparus*, and *Fasciola hepatica* can be distinguished using bead-based technologies for immunodiagnosing nematode infections [62]. However, like many immune-based assays, including those for haemonchosis, this test is genus-based rather than species-specific. Strategic deworming and infection management may benefit from the assessment of population immunity and the identification of flock members who are poor or nonresponders. Here, we discuss less common immunological assays that diverge from the detection of diagnostic markers derived from parasites, as well as immune-based advancements that concentrate on indirect, serological detection of haemonchosis. Therefore, the assays presented here investigate IgG and non-IgG antibodies against *Haemonchus* in addition to host responses to infection, including mast cell and mastocytosis, T cell proliferation, eosinophilia and eosinophil peroxidase, and changes in cytokine profiles as indicators of infection. Antibody tests such as Western blotting and ELISA are used to diagnose haemonchosis. Anti-*Haemonchus* antibodies that are specific to a given antigen can be found and measured using Western blot or ELISA. These methods entail immobilizing target antigens (whole parasite extract, secreted, purified native, or recombinant proteins) on a solid support and then exposing them to host bodily fluids (such as serum, mucus, and saliva) that contain antibodies specific to the antigen. After detection, a labelled isotype-specific secondary antibody is incubated, and then the proper substrate is added. Western blotting is frequently required to confirm specificity because ELISA testing is typically prone to nonspecific interactions. With isotype-specific antibodies, ELISA can measure all sheep immunoglobulins (Ig), including IgG, IgA, IgE, and IgM. The most extensively researched Ig classes in sheep infected with *H. cotortus* are serum or mucous IgG and IgA[63]. Radioimmunoassay was used in the early days to measure serum or mucous IgG and IgA [64]. However, ELISA quickly replaced Radioimmunoassay because it does not require the use of radioactive materials and has higher throughput and sensitivity. As previously mentioned, when the titer of specific IgG in sheep infected with *Haemonchus* is typically low and clinical symptoms

typically manifest before the antibody titres reach detectable levels, sensitivity and specificity can present challenges. More reagents have been available since the 1990s, allowing for the monitoring of *Haemonchus* antigen-specific IgG1 and IgG2, IgA, and IgM. Since IgG1 seems to be the most common antibody species triggered by *Haemonchus* infection, defining subclasses has become crucial[65]. Western blot, which first separates parasite antigens by SDS-PAGE, then transfers them to a membrane and screens them with diluted host antibodies, is another method for assessing antigen-specific antibodies in body fluids. It is not as efficient for high throughput and requires more labor, but it has the clear benefit of revealing whether antibody binding is cross-reactive or specific. If total antigens are used, this assay can also detect the presence of isotype-specific antibodies that clearly identify known antigen or antigens, or specific protein bands with known molecular masses. Like ELISA, this test has not been used exclusively as a haemonchosis diagnostic; nevertheless, it has emerged as a crucial instrument for *Haemonchus* discovery research[66]. Using antibody assays as research instruments to examine hemochosis Western blotting and ELISA are assays used to find *Haemonchus*-specific antibodies in bodily fluids, such as serum, tears, saliva, and feces from live animals. The literature has provided thorough descriptions of these assays [49], but there haven't been many noteworthy technological advancements mentioned. Although ELISA makes it easy to perform these assays, which typically target serum IgG, it can be difficult to detect IgE and IgA in the bloodstream, which are crucial for comprehending disease and are thought to be more significant than IgG when determining host protection levels. This is because expensive reagents are not readily available. It is possible to accurately measure antigen-specific IgE and IgA from infected animals in local mucosal. As a result, these tests are very useful for lab work but less useful for diagnosing haemonchosis in living animals. With its high sensitivity, accuracy, and reproducibility, capture/sandwich ELISA is typically used to detect low levels of IgE and IgA in blood. An IgA sandwich ELISA that is widely cross-reactive. The measurement of this Ig subtype by ELISA has been made possible by the availability of commercially available antibodies against sheep IgE [51] . Using the antisheep IgE monoclonal antibody, that produced from chimeric IgE protein, The sheep IgE capture ELISA was created [52] . This points of information that need for improvment and more sensitive assay to consistently measure IgE that found in body fluids and tissue homogenates of ruminants infected with *H. cotortus*, since IgE promotes basophil activation and IL-4/IL-13 release, both of which are critical for host protection against helminthes infections in the mouse models[67]. These antibodies should be more helpful in assessing population mucosal immunity and in the choosing animals with natural resistance to *H. cotortus* infection, which can be mediated by high levels of IgA and IgE, if key *H. cotortus* antigen specific IgA and IgE can be more reliably and precisely detected in ovine blood or other bodily fluids. Eosinophils and assays for eosinophil peroxidase Eosinophilia have been linked to protection and is well-documented in animals infected with *H. cotortus* as well as other nematode infections [68]. This cell population in whole blood has historically been counted to determine eosinophilia. However, it can be challenging to determine the postmortem eosinophil counts in the blood and tissues of infected animals, and the results of the enumeration assays currently in use are frequently very inconsistent and variable. Eosinophil-specific peroxidase levels that found in serum, tissue homogenates, and other bodily fluids can be measured by using sensitivity sandwich ELISA in recent developments to evaluate eosinophilia. Only the primary and secondary granules of mammalian eosinophils contain eosinophil peroxidase. Using a matched pair of monoclonal antibodies specific to Eosinophil-specific peroxidase this sandwich ELISA detects eosinophil activation, including degranulation, and may also be correlated with the degree of activation (e.g., number of activated eosinophils) [69]. Evidence linking eosinophil-specific peroxidase to host protection is lacking, even though it is a good indicator of eosinophilia induced by nematode parasites [70] . This assay's ability to identify ruminant eosinophil-specific peroxidase makes it a potential tool for evaluating both individual and population immunity as well as for choosing eosinophil-mediated resistant breeds. Mastocytosis and mast cell assays Mastocytosis, like eosinophilia, is associated with protection against infections caused by gastrointestinal nematodes, including *H. cotortus* [71]. Therefore, tryptase [72] and other local or systemic mast cell-specific markers can be used to measure mast cell activation/degranulation and to determine the host's susceptibility to parasites. There isn't currently an assay for the precise identification of sheep mast cell tryptase; Nonetheless, it seems that a bovine ELISA tryptase exhibits the broad cross-reactivity with tryptases for other hosts species, such as sheep and goat. Mast cell proteinase-1 is another indicator of infection; it is a serine proteinase with dual chymase/tryptase activity [72]. It is transported to the surface mucosa during nematode infections and expressed in gastrointestinal mast cells. Mast cell tryptase and sheep mast cell proteinase share functional There is an ELISA that targets SMCP [55]. Since sheep mast cell proteinase is higher in immune gastric mucosa than in normal tissues, its presence has been associated with protection in sheep infected with *H. cotortus* [55]. The application of sheep mast cell proteinase to live animals is limited because, despite being abundant in homogenates of abomasal

tissue of parasite-immune sheep, it is low to undetectable in serum and lymph. Moreover, the sheep mast cell proteinase antibody interactions may be disrupted by inhibitors found in sheep serum and lymph similarities in terms of fibrinogen cleavage and fibroblast stimulation [55]. There is an ELISA that targets sheep mast cell proteinase [55] . Since sheep mast cell proteinase is higher in immune gastric mucosa than in normal tissues, its presence has been associated with protection in sheep infected with *H. cotortus* [55]. The application of sheep mast cell proteinase to live animals is limited because, despite being increasing in homogenates abomasal tissue for parasite-immune sheep, it is low to undetectable in serum and lymph. Moreover, the sheep mast cell proteinase antibody interactions may be disrupted by inhibitors found in sheep serum and lymph. Consequently, homogenates from abomasal tissue are ideal for use with the sheep mast cell proteinase ELISA. Assays for cytokines and host alarming It is commonly known that cytokines play a role in the expulsion of gastrointestinal nematodes. The gastrointestinal epithelial cell reacts to give infection by releasing natural cytokines, including IL-1, IL-25, IL-33, and thymic stromal lymphopoietin in anticipation of the protective Th2 immunity against such nematodes. They also release molecules known as danger-associated molecular pattern or tissue/cell injury-associated alarmins, which include ATP, high mobility group box 1 (HMGB1), uric acid, and S100 proteins [73]. Delineating the pathways involved in the infection process can therefore be influenced by describing the animal's immune state. For instance, adenosine and ADP levels were found to be significantly lower during *Haemonchus* infection, even though one might anticipate an increase in ATP levels due to cell damage [59]. This result is consistent with the role that adenosine and ADP play at regulating of platelet activation and enabling blood feeding for *Haemonchus*.

Molecular methods for identifying *Haemonchus*

Although molecular diagnostic techniques offer novel alternatives for detecting the presence of *H. cotortus*, they are limited by the way faeces impede the polymerase chain reaction (PCR) and are not readily available to field veterinarians [9]. Droplet digital PCR has shown promise in the ongoing development of appropriate PCR tests . Molecular testing had the best sensitivity and could identify *H. contortus* down to the species level, whereas the McMaster approach of counting fecal eggs had a high specificity but a lower sensitivity. Numerous "first-generation" genetic and biochemical techniques have been developed over time to identify *Haemonchus* species and analyses genotypes that are resistant to drugs. Among the most popular first-generation technology examples were agarose gel electrophoresis and restriction enzyme digestion [74] , Southern blotting[75], repetitive DNA hybridisation probes in combination with Southern blots or dot blots[76], and isoenzyme banding profiles [77]. However, sensitivity and specificity were the main problems that led to the switch to PCR-based assays in order to create more sophisticated diagnostics. Additionally, DNA sequencing has long been a tool for distinguishing closely related species, but it only recently.

Research on the creation of quick with accurate molecular tests for the quantitative indicators of specific species found in faecal samples has been concentrated since the 1990s [50]. The ability to diagnose stages of parasitic worms that are similar and therefore challenging to distinguish through morphological examination has greatly increased with the advent of various molecular technologies, especially the polymerase chain reaction (PCR) methods based on parasite DNA examination With the advent of various molecular technologies, particularly the polymerase chain reaction (PCR) methods based on parasite DNA examination, the capacity to diagnose stages of parasitic worms that are similar and thus difficult to distinguish through morphological examination, has increased significantly. Molecular techniques not only have the potential to be more sensitive than microscopy, but they can also be automated and require less time than larval differentiation after fecal culture [74]. The molecular tests, LAMP and quantitative polymerase chain reaction (qPCR), seem to have a great deal of promise to advance our diagnostic skills in this field and provide insight into whether nematode cross-transmission happens between domestic and wild ruminants and whether it plays a major role in the emergence of resistance on livestock farms. Furthermore, extracellular HMGB1 has been demonstrated to have a dual function, acting as a secreted molecule from activated immune cells or a passively released molecule from damaged cells, regulating both inflammation and cellular repair. Recently, molecular phylogenies of the phylum Nematoda have been proposed, based on the internal transcribed spacer 2 (ITS2)[78]. Until recently, only the family Trichostrongylidae and the superfamily Trichostrongyloidea [79] had been the focus of attempts to create a phylogenetic classification of the group using cladistic methods. The ITS1 and ITS2 regions of rDNA have been typically used for diagnostic purposes [80] with some phylogenetic reconstructions restricted to the intragenic level. Molecular studies have been common among these groups [81]. Using the mitochondrial DNA cytochrome oxidase subunit I gene (mtDNA COI) sequences, the genetic variability of *H. contortus* from sheep and goats showed high rates of gene flow between populations. The suborder Strongylina was the sole focus of a significant high-level molecular phylogenetic analysis of the Strongylida based on ITS2 sequences . High genetic variation and comparatively low host specificity for *H. contortus*

were reported in Brazil and Italy by other studies that focused on both domestic and wild animals[19]. Australia, Brazil, Europe, Malaysia, and the United States are just a few of the topographical locations where population genetic studies of *H. contortus* have been carried out[82]. To the best of our knowledge, however, *H. contortus* genetic variability in Egypt has not yet been considered.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

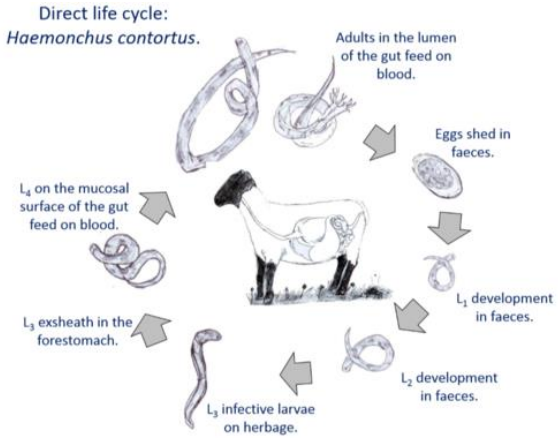

Fig.1. *Haemonchus Contortus* Life Cycle

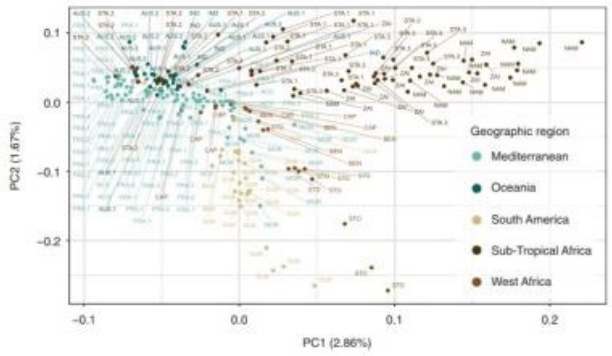

Fig. 2. Global diversity of *H. cotortus* isolates. Principle component analysis of genomic diversity. It is based on genotype like hood inferred from whole genome sequences of 223 males (243012 variants considered). Available from: Nature Communications This content is subject to copyright. Terms and conditions apply.

TABLE 1.summary of epidemiological features of *H. cotortus* infections according to climatic changes

Climatic Zones	Regions	Ecological Features	Parasite Epidemiology
Tropical and subtropical regions	Tropical regions of Africa and America, tropical islands of the Pacific Ocean, southern and south-east Asia, northern part of Australia, southern USA, the Caribbean	L_3 not surviving on pasturelands for long: moisture permitting larval development during dry seasons; longer time of larval survival and in cold, potential survival development when adequate moisture	Larval populations are continuously developing, and animals are persistently under stress; during dry conditions, L3 numbers rise seasonally; hypobiotic L4 emerge during dry periods.
Worm temperature and summer rainfall regions	Parts of southern USA and South America , southern and eastern Asia, southern Africa, eastern Australia	The combination of elevated temperatures and moisture promotes the growth of L3; however, under colder conditions, the survival and development of larvae slow down.	Major issue, influenced by rainfall; when winter temperatures are moderate, L3 may occur year-round; in regions with colder temperatures, outbreaks vary with the seasons; hypobiosis is more common during harsh winters.
Mediterrane an climatic regions	*South west cape of South Africa, para-Mediterranean basin, south east Australia, western Australia*	Halt in survival and growth of independent life stages; larval populations' peak during autumn and spring; during warmer winter temperatures. Potential survival of L3.	Peak populations occur from late autumn to early winter and from late spring to early summer; differing hypobiosis aligns with the length and severity of hot and dry conditions.
Cool and cold temperate regions	Northern USA and Canada, New Zealand, south east-Australia, northern Europe	Larval development is stopped until the weather become more temperate.	Minimal danger.restricted to warmer m onths; hypobiosis allowing for overwin tering; hypobiotic larvae developing qu ickly
Arid regions	Deserts of southern and sub-Sharan Africa, continental Australia, Middle East	Larval population growth and s urvival are limited by a lack of moisture, which is preferred dur ing times of rains.	Larval availability is negligible; hypobi osis of variable significance lowers L3.

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عدوى هيمونكس كونتورتس في الأغنام مع التركيز على الأنيميا، الوبائيات والتشخيص

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الملخص

داء الديدان المعدية هيمونكوسس هو مشكلة عالمية تؤثر على الأغنام يعيش الطفيلي شديد الضراوة هيمونكس كونتوريس في الأمعاء الدقيقة للحيوانات المصابة ويسبب المرض عن طريق امتصاص الدم، مما يؤثر سلبًا على إنتاجية الحيوانات وصحتها العامة .المشكلة الأولى هي أن العدوى ليست موزعة بالتساوي في جميع أنحاء العالم؛ فهي أكثر شيوعًا في المناطق المعتدلة والاستوائية وشبه الاستوائية الدافئة ذات الأمطار الصيفية مقارنة بالمناطق الباردة والجافة. ويُعد سببًا رئيسيًا للوفاة. تستعرض هذه المراجعة الأنيميا (فقر الدم) وعلم الوبائيات وطرق التشخيص من خلال التعرف المورفولوجي والفحص المجهري والتقنيات المناعية والجزيئية. تعتمد نتائج العدوى المحتملة على شدة الإصابة والاستجابة المناعية للأغنام، مع أن العدوى قد تكون قاتلة في بعض الحالات. لذلك، يتم في هذه المراجعة مناقشة الأدبيات المتعلقة بفقر الدم الناتج عن هيمونكوزسس والأعراض السريرية المصاحبة للعدوى المفرطة الحادة، الحادة، والمزمنة بشكل مفصل. كما يتم. يتم تقييم تقنيات التشخيص المختلفة مثل التعريف المورفولوجي والفحص المجهري، كما يتم تقييم الطرق المناعية والجزيئية.

الكلمات الدالة: داء الديدان المعدية، الأنيميا، التشخيص المناعي، التشخيص الجزيئي.