



Parasitological Prevalence of *Cryptosporidium* infection in calves, chickens and human in Behera province

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Abstract:

Cryptosporidium is a cosmopolitan intestinal protozoan parasite that belongs to the phylum Apicomplexa and infects a broad range of vertebrate hosts including livestock, human and birds. This current study was aimed to investigate the prevalence of *Cryptosporidium* spp. infection in young calves, human and chickens, in five districts covering five different geographical locations of the Behera province. In doing this, a total number of 310 faecal samples from calves (n= 148), human being (n=112) and chicken (n=50) were collected, stained using the modified Ziehl-Neelsen staining method, and were examined microscopically for *Cryptosporidium* spp. oocysts. Correlations between prevalence rates and related parameters such as species, age, gender and faecal consistencies were also analyzed. Results indicated that 43.2%, 16.1% and 6%, of calves, human and chicken, were found infected with *Cryptosporidium* spp., respectively. Statistically significant variable rates of infection were also detected across six surveyed districts of the Behera governorate, with the notable absence of infection from chicken in Abo Hummus and Rahmaneyah districts. Statistically significant variable rates of infection were also characterized in calves when species, age, sex, and faecal consistency were analyzed; buffalo >4 weeks calves, male and calves with formed faeces have higher infection rates than their counterparts of cattle <4 weeks, female, and calves with semi-formed and diarrheic faeces. Slightly contrast data were obtained from human; younger (<15 years), female, and diarrheic individuals have higher infection rates than their compared counterparts. Detection of *Cryptosporidium* spp. in the livestock and chicken living in close proximity to human population necessitates better surveillance and control measures to protect vulnerable animal and human populations.

Key words: *Cryptosporidium*; calves; human; chicken; modified Ziehl-Neelsen staining

Introduction

Cryptosporidium is common enteric parasite that belongs to phylum Apicomplexa. It is a coccidian protozoan infects a wide range of vertebrate animal hosts, human and birds (Fayer, 2010; Plutzer and Karanis, 2009 and Ryan et al., 2014).

In cattle, infection with *Cryptosporidium* is prevalent in the pre-weaned calves, resulting in a clinical disease with the potential of zoonotic transmission to human contacts, particularly in the case of *C. parvum* (Feng et al., 2007 and Zhang et al., 2013). In humans, cryptosporidiosis results

in many diarrheal outbreaks globally and may be acquired through direct contact with infected person or animals, and indirectly through consumption of food contaminated with oocysts (**Chalmers and Davies, 2010** and **Xiao, 2010**). Water also represents a major source of infection as *Cryptosporidium* oocyst is considered resistant to chlorine used in water disinfection (**Baldursson and Karanis, 2011** and **Karanis et al., 2007a**). *Cryptosporidium* is estimated to be the second leading etiological infectious agent of diarrhea and death in infants in poor countries after rotavirus infection (**Kotloff et al., 2013** and **Striepen, 2013**). In immunocompetent persons, the clinical picture of the disease is usually slight fever, mild abdominal discomfort and self-limiting watery diarrhea, but in immunodeficient humans, however, the infection is frequently chronic with persistent diarrhea, malabsorption and may lead to death, especially in the Acquired Immuno Deficiency Syndrome (AIDS) and cancer patients (**Wumba et al., 2015**).

On the other hand, birds are infected with a number of *Cryptosporidium* species. Chicken, turkeys and aquatic birds are considered as a reservoir for transmission to human particularly in the case of *Cryptosporidium meleagridis* (**Bomfim et al., 2013**).


Cryptosporidium infection in farm animals and humans is detected through several methods that can be broadly categorized under microscopy, serology, and the molecular-based diagnostic methods (**Khurana and Chaudhary, 2018**). Despite advances that have been made in developing serological and molecular tools of *Cryptosporidium* diagnosis such as ELISA, IFAT, PCR-RFLP, the microscopical observation of oocysts in the stool/faecal samples using acid fast stains like the modified Ziehl–Neelsen stain and Kinyoun stain remains the most conventional and the gold-standard technique of cryptosporidiosis

diagnosis (**Clarke and McIntyre, 2001** and **Ma and Soave, 1983**).

In Egypt, the intensive and semi-intensive farming practices of the cattle livestock in the highly dense-populated delta region constitute a major source of infection dissemination (**El-Saharty et al., 2005**). Similarly, keeping the livestock in close proximity to human and other animal species and birds is major zoonosis concerns, making the monitoring of occurrence of *Cryptosporidium* in human and animals a priority from the perspective of the veterinary and public health importance. Thus, this study was initiated with the aim of surveying the occurrence of *Cryptosporidium* in calves, human, and chicken, by examining faecal/stool samples that were collected from overlapping geographical areas located at the Behiera province. Correlation between different factors, such as species, age, sex and the faecal consistency, with that of infection prevalence rates was also assessed.

Material and Methods

Study locations:

The study was conducted in the municipalities of Behera province, located in the West of the Delta region of Egypt (Coordinates:  30.61°N 30.43°E). Five locations representing five districts were sampled during the current study, covering the South, West, North and the East of the province. This namely included Delingat, Shubrakhit, Rahmania, Abou Hommos and Hosh Eisa (**Fig.1**).

Animals and human being and sample collection:

During the period from January to October (2019), a total of 310 fresh faecal samples were collected from calves, human and chickens, respectively. These included 148 pre-weaned calves of ages ranged from one week to up to 2 months, different sexes of cattle versus buffaloes, and of different faecal consistency; diarrhoea, semi formed and formed. For calves; faecal samples were obtained

directly from the rectum of each individual animal. For human; stool specimens were collected from 112 individuals attending various health units (hospitals, rural clinics and private diagnostic laboratories), as these individuals were of different ages, gender and stool consistency. For chickens; pools of faecal droppings were collected from 50 chicken flocks aged over 90 days (the minimum number is 10 birds /flock) from areas that overlapped with calves and human being.

Collection and identification of oocysts:

Faecal specimens were collected in plastic cups, immediately transported in ice -cooled to the laboratory of Parasitology Department, Faculty of Veterinary Medicine, Alexandria University. If faecal specimens unable to be examined directly, the specimens were kept with 2.5% potassium dichromate solution in refrigerator at 4 °C according to **Karanis et al.(2007b)** and **Ma et al. (2014)**. Direct smears were prepared from faecal specimens and processed for microscopical examination. Also, the oocysts were identified by acid fast staining by using modified Ziehl-Neelsen stain according to (**OIE, 2008**) for more clarify and identifications. Briefly, about 1gm of faeces were transferred to a clean microscopic slide, thin smear was done with another glass slide spreader. Then the smears were dried in the air or in hot air oven under 40-50 C and fixed with few drops of absolute methanol for 5 minutes. The slides were then stained with carbol fuchsin for 15 min, and the excess stain was washed with tap water before they were decolorized with 1% acid alcohol for 15 seconds. The slides were washed again with tap water before counterstained with 0.4% methylene blue for 1 min, and before finally rinsed with tap water and air dried. *Cryptosporidium* oocysts were detected by examining the stained smears under the 40X and 100X-oil immersion objectives of the optical light microscope (Optika, Italy).

Statistical analysis:

The potential statistical correlation between prevalence and various parameters (animal host species and the geographical locations), and the correlation between the detection rates within the same host species in relation to parameters of age group, sex and fecal consistency was done. This was done by using the Chi square test and the Fisher's test included in the SPSS statistical package. Statistical significance was proved when p-value was < 0.05.

Result

Microscopy-based examination of faecal smears stained with the modified Ziehl-Neelsen stain revealed the characteristic diagnostic stages of *Cryptosporidium* as acid fast-stained oocysts on a blue background (**Fig. 2**). The overall prevalence rates of *Cryptosporidium* spp. in calves, human being and chickens were 43.2%, 16.1% and 6%, respectively (**Table 1**).

With except of chickens, infection with *cryptosporidium* was recorded from calves and humans in all the five districts assayed, albeit with no statistical significance (P=0.431) of detection rates between different districts (**Table 2**). When placed in comparison, Hosh Eisa was recorded with the highest rate of positive calves (57.9%), but with the lowest rate of human with *cryptosporidium* infection (3.1%). This is completely opposed to Rahmaneyah, with the highest and lowest rates were for human and calves, respectively (**Table 2**).

Cryptosporidium infection was detected in calves of different species, age, sex, and the faecal consistency status (**Table 3**). With except of species, statistically significant differences were recorded with age (P=0.016), sex (P=0.023) and faecal consistencies (P=0.047). With species, 28 out of 80 (35%) of cattle calves and 36 out of 68 (52.9%) of buffalo calves, were recorded positive with *cryptosporidium*, albeit with

no statistical significance ($P=0.112$) of the difference between the two species. Older calves (> 4 weeks) have had a higher statistically significant incidence rate of *cryptosporidium* (44.4%) than younger calves (< 4 weeks) rate (40.4%). Results also revealed that 45.5% (50 of 110) of male and 36.8% (14 of 38) of female calves were positive for *Cryptosporidium* (Table 3). *Cryptosporidium* was detected in calves with formed, semi formed and watery faecal conditions by infection rates 47.9%, 31.6% and 42.9%, respectively.

Similar to calves, variable detection rates for *cryptosporidium* were recorded from human of different age groups, gender and stool consistencies, albeit in a non-statistically significant variations (Table 4). *Cryptosporidium* infection was detected in 17.2% (10 of 58) and 14.8% (8

of 54) in age groups of <15 years and > 15 years, respectively. From the gender perspective, 11.5% (6 of 52) of male persons and 20% (12 of 60) of females were identified as positive for *Cryptosporidium*. Similarly, diarrheic human persons registered a higher detection rate (35.7%) than those with formed (10%) and semi-formed (9.1%) stools (Table 4).

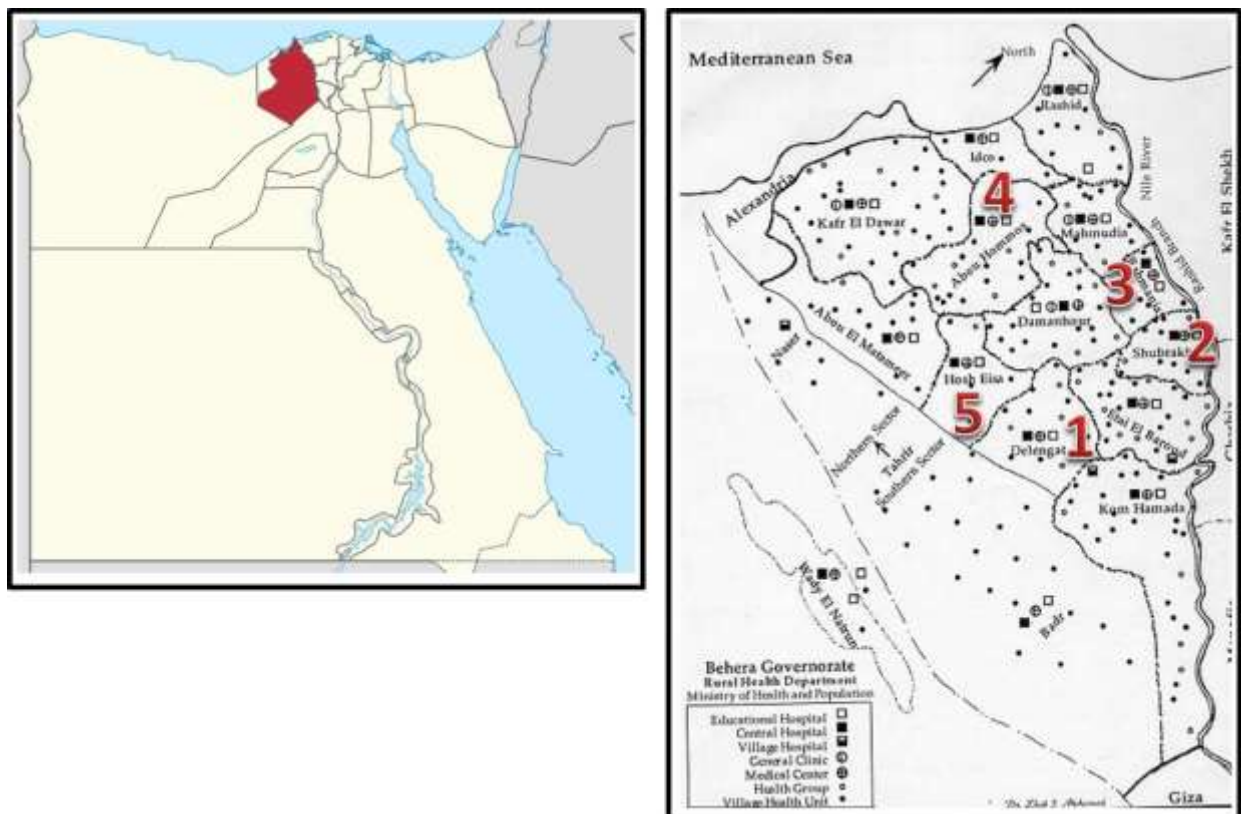


Fig. 1: A map showing the location-sampling sites for the current study in Behera governorate.

(1) Delingat, (2) Shubrakhit, (3) Rahmania, (4) Abou Hommos, and (5) Hosh Eisa

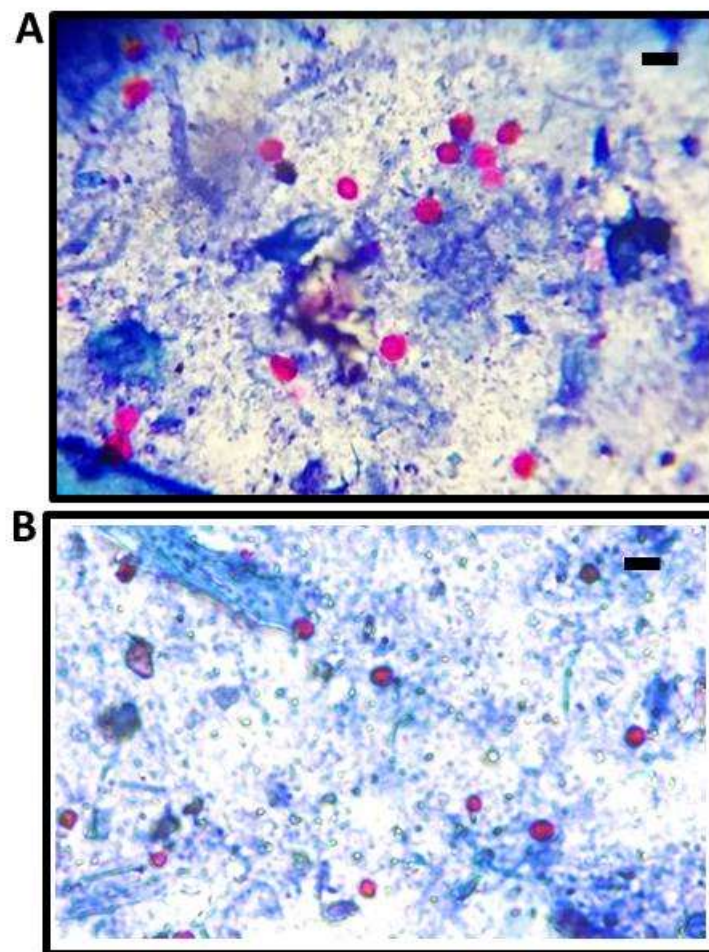


Fig. 2: Detection of oocysts of *Cryptosporidium* spp. in the faecal smears that were processed using the modified Ziehl-Neelsen staining. A) Mammals (calves, human being); B) Chickens. Microscope magnification of 40X. The scale bar: 5 μ M.

Table 1: Prevalence of *Cryptosporidium* spp. in the faecal/stool smears of calves, humans and chicken

Host	Number of examined samples	Positive	Percentage (%)	95% CI	p-Value
Calves	148	64	43.2	2.5-17.2	0.139
Human	112	18	16.1	2.3-17.8	
Chicken	50 (pools)	3	6	2.3-19.9	

Table 2: Prevalence of *Cryptosporidium* spp. in calves, humans and chicken according to the geographical locations of the sampling sites

Region	Calf		Human		Chicken		p-Value
	Examined samples (n)	No. Positive samples (%)	Examined samples (n)	No. Positive samples (%)	Examined samples (pools) (n)	No. Positive samples (%)	
Delengat	26	9 (34.6)	18	4 (22.2)	12	1 (8.3)	P=0.431
Shubrakhit	38	13 (34.2)	14	2 (14.3)	9	1 (11.1)	
Abu Hummus	19	11 (57.9)	21	5 (23.8)	6	Zero (0)	
Hosh Eisa	42	24 (57.1)	32	1 (3.1)	16	1 (6.2)	
Rahmaneyah	23	7 (30.4)	27	6 (22.2)	7	Zero (0)	

Table 3: Detection rates of *Cryptosporidium* spp. in calves, and its relation to parameters of age, sex and fecal consistency

Groups	No. of examined	No. of positive (%)	95% CI	p-Value
Species				P=0.112
Cattle	80	28 (35)	10.3-33.5	
Buffalo	68	36 (52.9)	9.9-38.7	
Age (weeks)				P=0.016
1-4	58	24 (41.4)	3.6-13.7	
4-8	90	40 (44.4)	5.0-27.7	
Sex				P=0.023
Male	110	50 (45.5)	7.2-22.5	
Female	38	14 (36.8)	4.2-9.4	
Fecal consistency				P=0.047
Diarrhea	14	6 (42.9)	5.3-41.2	
Semi-Formed	38	12 (31.6)	6.2-29.2	
Formed	96	46 (47.9)	9.0-7.0	

Table 4: Detection rates of *Cryptosporidium* spp. in the human and its relation to parameters of age, gender and fecal consistency

Groups	No. of examined	No. of positive (%)	95% CI	p-Value
Age (years)				
<15	58	10 (17.2)	3.0-13.6	P=0.126
>15	54	8 (14.8)	3.2-11.5	
Gender				
Male	52	6 (11.5)	3.3-16.0	P=0.127
Female	60	12 (20)	3.4-14.7	
Stool consistency				
Diarrhea	28	10 (35.7)	5.7-42.2	P=0.077
Semi-formed	44	4 (9.1)	5.4-44.6	
Formed	40	4 (10)	5.5-43.8	

Discussion

Comparing the current results with that previously recorded from calves in Egypt; revealed a higher rate (43.2%) than previously recorded rates of 30.2%, 34.1%, 30.4% and 9.2% of *Cryptosporidium* infection in calves in different Egyptian governorates (Amer et al., 2010; El-Seify et al., 2012; Ghoneim et al., 2017 and Shaaban et al., 2018). As well as the current study revealed *Cryptosporidium* infection in human was 16.1% and was lower than that found by (Ghoneim et al., 2017) who reported infection rate of 27%, but it was higher than (Helmy et al., 2013) who recorded 6.7% prevalence rate.

Only three districts out of five were presented with positive detection rates of *cryptosporidium* oocysts in chicken samples, with chicken from Abu Hummus and Rahmaneyah were *cryptosporidium*-free. No correlation overlapping was reported when the prevalence rates of three species were recorded across the same district. The

absence of the statistical significance and the correlation between prevalence rates, species examined, and districts of the study is in contrast to previously reported findings (Squire et al., 2017), who found a high correlation between species examined and the district location.

Rates recorded in this study were higher than previously recorded rates of 30.2% and 14.5% in the dairy and buffalo calves in Egypt, respectively (Amer et al., 2010 and El-Khodery and Osman, 2008). Older calves have had a higher infection rate than younger calves. Nevertheless, these current rates in the pre-weaned calves still much higher than previously recorded rates in the pre-weaned and post-weaned calves from Egypt (Amer et al., 2010; Amer et al., 2013 and El-Khodery and Osman, 2008). This difference in the prevalence rates between pre-weaned and post-weaned calves and between this study and previous ones might have been

attributed to different health, management, host, and environmental factors (Fayer et al., 2010 and Feng et al., 2012).

Our current data revealed that male calves have had higher prevalence rates than females. This is in contrast to the higher female than male calves recorded respectively (Squire et al., 2017). As also observed in this study, *Cryptosporidium* was detected in calves with formed, semi formed and watery faecal conditions, indicating that infection with *Cryptosporidium* could be latent and found in young calves without demonstrating any clinical signs (Fayer et al., 2005 and Wang et al., 2011). Therefore, it is essential to consider the asymptomatic *Cryptosporidium* infection that may result in reduced feed efficiency rates, decreased weight gain, with the ultimate loss in the dairy industry (Anderson, 1987; Esteban and Anderson, 1995 and Ralston et al., 2003).

In contrast to data of calves, females of human being were presented with a higher prevalence rate, which is similar to previously recorded data from Nigeria (Anejo-Okopi et al., 2016). Similarly, diarrheic human contacts registered a higher detection rate than those with formed and semi-formed, as in contrast to data in calves. These variable findings between calves and human most probably attributed to different species of *Cryptosporidium* infecting two host species (El Kader et al., 2012 and Helmy et al., 2013).

The overall infection percentage that was recorded in chicken (6%) was lower than rates recorded from free-range chickens with *cryptosporidium* infection in Brazil (25.6%) and layers flocks in

Germany (8.3%) (Ewald et al., 2017 and Helmy et al., 2017). Nevertheless, variation in the detection techniques between our current study (microscopy) and these of Brazil and Germany (PCR and DNA sequencing) might help to understand the variable detection rates. Since no parameters such as age groups, gender, species, and faecal consistencies were recorded in chickens, it become impossible to proceed further with analysis similar to that of calves and human. However, the findings of oocysts of *cryptosporidium*, and its potential zoonotic transmission to human and other mammals (Bomfim et al., 2013 and Majewska et al., 2009) add to the significance of the current data.

In conclusion, the current data indicated that *Cryptosporidium* infection is common in calves, and less extent to chickens. The lack of host specificity between mammalian species of *Cryptosporidium* (calves in this case) could be a potential natural reservoir host and risk of zoonotic transmission for human contacts in the Behera province. Also the presence of infection in the non-diarrheic calves and personnel further consolidated the significance of latent non-symptomatic infection sources for non-infected hosts, and insisted on the importance of routine examination in the endemic areas. From the future prospective, identification of implicated *Cryptosporidium* species by molecular methods, and examination of the potential evolutionary relatedness which could be inferred from the phylogenetic analysis become increasingly important to gain better understanding of the zoonotic potential of this parasite between mammalian hosts.

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الملخص العربي

الانتشار الطفيلي للعدوي بالكريبتوسبورديوم في العجول والدجاج والانسان في محافظة البحيرة

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الكريبتوسبورديوم هو طفيل عالمي معوي ينتمي إلى فصيلة Apicomplexa ويصيب مجموعة واسعة من الفقاريات بما في ذلك الماشية والانسان والطيور. ولقد أجريت هذه الدراسة لمعرفة مدى انتشار طفيل الكريبتوسبورديوم في العجول الصغيرة، الانسان وكذلك الدجاج في خمس مناطق تغطي خمسة مواقع جغرافية مختلفة من محافظة البحيرة. وعليه فقد تم تجميع ٣١٠ عينة براز وشملت علي ١٤٨ عينة من العجول، ١١٢ من الإنسان و٥ من الدجاج، وقد تم معالجة جميع العينات سالفة الذكر بصبغة Ziehl-Neelsen المعدلة، وفحصت مجهرياً لرؤية الطور المعدي لطفيل الكريبتوسبورديوم. كما تم تحليل العلاقة بين معدلات الانتشار والعوامل المختلفة مثل النوع والعمر والجنس ونوعية البراز. وقد أشارت النتائج إلى أن ٤٣,٢%، ١٦,١% و ٦% من العجول والانسان والدجاج، على التوالي، كانت إيجابية لعدوى الكريبتوسبورديوم. كما تم اكتشاف معدلات متفاوتة للإصابة في الخمس مناطق التي شملتها هذه الدراسة، مع غياب ملحوظ للعدوى من الدجاج في منطقتي أبو حمص والرحمانية. كما أظهرت النتائج الي أن معدلات الإصابة في عجول الجاموس الأكبر من ٤ أسابيع، والذكور والعجول ذات البراز المتماسك أعلى من نظرائهم من العجول الأصغر من ٤ أسابيع، والإناث، والعجول المسهلة. وقد سجلت النتائج أيضاً أن معدلات الإصابة في الأشخاص الأصغر سناً (أقل من ١٥ سنة)، والإناث، والذين يعانون من إسهال أعلى من نظرائهم. وبناء علي هذه النتائج التي تم التوصل اليها واكتشاف طفيل الكريبتوسبورديوم في الأشخاص المخالطين للماشية والدجاج يتضح أنه لا بد اتخاذ إجراءات وقائية ومراقبة أفضل لحماية الحيوانات والبشر المعرضين للخطر.