Echinococcosis and research in Uzbekistan

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ABSTRACT
Over the past 10 years, the numbers of cases of human echinococcosis in Uzbekistan has increased by a factor of 3.7 and, in some regions, the annual human incidence is now greater than 10 cases per 100,000 population. In farm livestock and dogs, the prevalence of infection has increased substantially in the same time period. The prevalence in sheep is now over 62%, that of cattle greater than 42%, goats 11%, camels 35%, and donkeys 38%. Prevalence rates in dogs are generally about 28% in rural areas. Experimental infections in dogs suggest that the prepatent period of *Echinococcus granulosus* varies with the season of the year: the longest prepatent period (about 90 days) being in the winter, the shortest (about 31 days) being in the summer. We recommend that, in areas of high livestock infection, dogs should be treated with anthelmintics 8 times per year. We have tested the efficacy of a number of anthelmintic products to find the cheapest effective preparation for use in Uzbekistan. We also recommend that all infected stock should be incinerated when slaughtered to prevent transmission to dogs.

Эхинококкоз и его изучения в Республике Узбекистан

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РЮЗЮМЕ
Республика Узбекистана относится к числу эпидемических зон по эхинококкозу. Рост эхинококкоза за последний 10 лет составляет 3,7 раза.. Пораженность эхинококкозом среди крупного рогатого скота вырос в два раза, мелкого рогатого скота - в полтора раза, собак - почти в два раза. Установлена различия в сроках развития эхинококка в кишечнике собак в зависимости от сезонов года, кормления, содержания и их возраста в пределах одной республики. Выявлено, что развития эхинококков в кишечнике собак в условиях Республики летом завершится к 31-му дню, а зимой 83-му дню. С учетом биологии эхинококка разработана новая технология дегельминтизации собак, состоящая из преимагинальной и имагинальной. Сочетание этих двух дегельминтизации, проводимые 8 разов в год, обеспечивают быстрейшего оздоровления хозяйств и населенных пунктов от эхинококкоза. Разработанная новая технология дегельминтизации собак лечебными гранулами, содержащих антгельминтики оказался малотоксичной, дешевой и приемлемой во всех условиях ведения животноводства Республики. Парэнтеральное применение антгельминтиков против эхинококкоза собак обеспечивает полное изгнание гельминтов из организма собак. Предложенные в практику бетонные резервуары для обеззараживания пораженных органов эхинококкозом животных и бетонные печи для сжигания бракованных органов, являются приемлемым и в условиях пустыни, гор и отгонного овцеводства.

INTRODUCTION

Echinococcosis is a serious and widespread disease causing serious human health problems and economic damage to the livestock industries in Uzbekistan. According to data from the Ministry of Health, during the period 1988-2001,11,239 people were diagnosed with the disease. During the last 10 years the number of people infected has increased by 3.7 times.

Nazirov (2002) reports that between 1999 and 2001, 4,109 people were operated on for echinococcosis. The analysis of the data shows that echinococcosis is frequently distributed amongst children and young adults. In 2000, 14.3% of cases were in children less than 14 years of age. Of the 85.7% adults, 54% were women and 46% men. In 2001, proportion of paediatric cases had risen to 20.4 % whilst of the adult cases 50.9% were women and 49.2% men. Between 20% and 30% of cases had recurrences after surgery, whilst surgical case mortality rates were was between 2% and 5%. Boimuratov (1996) analysed 86 paediatric case histories from the children's surgical hospital in Sammarkand. Of these cases 27.9% were in children less than 7 years of age, 25.6% in children of 8-10 years and 46.5% in children 11 -15 years of age. The overall incidence rates for human echinococcosis are highly variable, with very high levels (> 10 cases/100,000) recorded in Bukara and Sirdarin Oblasts and less than 1 case per 100,000 in Tashkent and Djinzal Oblasts. The regional incidence of echinococcosis is detailed in Table 1.

Table 1. The regional incidence (annual cases pre 100,000 population) of Uzbekistan.

Oblast	Incidence (cases per 100,000)
Andijan	2.90
Buhara	11.81
Dj inzak	0.76
Kashkadarini	7.46
Navoin	5.67
Namangent	5.28
Samarkand	3.67
Surhandarin	6.35
Sirdarin	10.16
Tashkent	0.15
Fergan	9.39
Horezm	14.55
Tashkent City	1.68
Uzbekistan	1.53

Paralleling the rise in the incidence of human echinococcosis has been an increase in the prevalence of echinococcosis in animals. Between 1990 and 2002, the prevalence rate in sheep has increased from 45% to 62% whilst in cattle it has increased from 24% to 45%.

Data of the prevalence rates in agricultural animals and dogs is presented in Table 2.

Recently we have undertaken a study of the prevalence rates in dogs by purging 531 dogs with arecoline from 9 different townships. Both village dogs and farm dogs were investigated. The results are detailed in Table 3.

Prevalence rates varied quite considerably, but farm dogs were significantly more heavily infected than village dogs overall which is consistent with findings from a similar study in Kazakhstan (Torgerson *et al* 2003). The widespread infection df dogs in Uzbekistan indicates the low level of anthelmintic prophylaxis used or the poor efficacy of arecoline. Even when

successfully purged, an infected dog can still contaminate the environment for 36 hours. New preparations such as praziquantal, cestel, kanikbantel and others, which have better efficacy than arecoline, have become available but are too expensive for widespread usage.

Table 2. Prevalence rates in domestic livestock and dogs between 1990 and 2002.

	Prevalence rates						
	1990	1993	1996	1999	2000	2001	2002
Cattle	24.3	27.6	26.2	31.0	36.3	38.2	45.5
Sheep	45.1	48.3	54.5	57.0	59.1	61.0	62.2
Goats	8.0	8.7	9.3	10.1	9.0	9.5	11.1
Pigs	6.0	6.5	8.0	—	—	—	—
Camels	25.4	28.3	31.0	29.4	31.1	33.0	35.0
Donkeys	32.0	34.3	37.1	36.5	36.4	38.0	38.5
Dogs	15.0	18.4	18.9	20.1	22.5	24.5	28.6

Table 3: The results of a study where 531 were purged with arecoline from 9 different areas of Uzbekistan

Study	Total investigated	Number infected	x©	Farm Dogs			Village dogs		
				Total investigated	Number infected	X© O⁴	Total investigated	Number infected	x© o⁴
Mulk	265	35	13.2	134	23	17.1	119	12	10.0
Saxoba-ota	89	15	16.9	15	12	80.0	74	3	4.0
Olga	52	9	17.3	34	7	20.6	18	2	11.0
Upus	15	3	20.0	15	3	20.0	-	-	
Kattakurgan	13	2	15.4	13	2	15.4	-	-	
Amasai	27	4	14.8	27	4	14.8	-	-	
Darvoza	7	1	14.2	7	1	14.2	-	-	
Erembe Tova	12	2	16.7	12	2	16.7	-	-	
Amangeldi	51	4	7.8	22	2	9.0	29	2	6.8
Total	531	75	14.2	279	56	20.1	240	19	7.9

SEASONAL DEVELOPMENT OF *ECHINOCOCCUS* IN THE DEFINITIVE HOST.

There are a variety of opinions in the literature regarding the development time of *Echinococcus* in dogs with suggested prepatent periods of between 24 and 90 days. (Sibold 1853, Kuchenmeister 1855, Yamashita *etal* 1958, Reddy & Suvamarumari 1971,Bandai 1948, Nosik 1953, Gorina 1962, Zenkov & Slepnev 1974, *Razakov et al* 1986). The variance in the reported development time seems to depend upon the country in which the studies were undertaken. Reddy & Suvamarumari (1971) reported in India that the development of the parasite is completed by day 83. However, Pandgy (1972) asserts that patency begins after 42 days. Zenkov and Slepnev (1973) in Belarus observed, that full development of the cestode

occurs between 43 and 66 days after infection. In our opinion, fluctuation in the development of *Echinococcus* in the intestines of dogs depends on many factors, which have not been studied sufficiently. These include the age of dogs, how they are maintained, seasonal variations and climatic conditions. Some of these factors may accelerate development whilst others may slow development down. Therefore a worming programme may need to be varied between countries or even within one country depending on these factors. Consequently, we have studied the development of *Echinococcus* in the intestines of dogs in conditions ecountered in Uzbekistan in the winter, summer and in the autumn.

A total of 12 dogs were each experimentally infected with 25,000 viable protoscolices. The dogs were all of similar age and breed. There were 3 experimental groups of 4 dogs. One group was infected in the summer, one in the autumn and one in the winter. Faecal examinations were started at 25 days of infection and were continued on a daily basis until all dogs in the group had eggs and proglottids in the faeces. The dogs were then euthanised and the adult parasites were recovered from the small intestine. The total number of parasites were enumerated, the sizes of the parasites from a representative sample from each group of dogs was determined and the morphology and sexual maturity of the proglotids determined. The results are summarised in Table 4.

In the group of 4 dogs infected in the summer, eggs were first detected in the faeces of 1 dog 31 days after infection and by 35 days after infection all animals had patent infections. At necropsy, on day 35 a total of 78,318 adult parasites were recovered with a mean of 19,579.5 from each dog (Table 3). The detailed morphology of 535 adult parasites recovered from these dogs was investigated. Of these, 55.6 % had three proglotids whilst 44.4 % had 4 proglotids. The length of the strobila ranged from 2.040 up to 3.0 mm, whilst the length of the last proglotid ranged from 0.990 to 1.950 mm. The last proglotid was filled with mature eggs. These data indicate that in the summer in Uzbekistan *E. granulosus* reaches sexual maturity by 31 days after infection.

In the second experiment of 4 dogs infected in the month of September, eggs were detected in the faeces at between 39 and 43 days after infection. At post-mortem, 45 days after infection, 47,697 adult *E. granulosus* were found, with an average of 11,924 in each dog. Detailed examination of 283 parasites demonstrated that 11.2% had 2 proglotids, 66.6% had 3 proglotids and 22.2% had 4 proglotids. The length of the strobila were between 4.02 and 5.394 mm, with the length of the last proglotid between 2.215 and 3.018 mm. The uterus was filled with mature eggs. This data suggests that the prepatent period of *E. granulosus* is 39 days in the autumn.

In the dogs infected in the winter, the first eggs and proglotids were observed in the faeces on the 83rd day after infection. The dogs were necropsied at 97 days after infection when all dogs had patent infections. A total of 41,638 parasites were recovered with an average of 10,409 in each dog. Of 256 parasites examined in detail, 91.6 % had three and 8.4 % had four proglotids. The lengths of the strobila were between 3.534 and 4.253 mm, and the length of the last, mature proglotid was 1.612-2.046 mm. The last proglotid was filled with mature eggs.

These experiments suggest that the prepatent period can vary considerably between summer and winter. In winter, it is 52 and 44 days longer than in summer and autumn respectively. Therefore, the period between anthelmintic treatment can be varied accordingly. This data also suggests that the size of the mature parasites vary according to season, being largest in autumn and winter and smallest in summer.

Table 4. Development of *Echinococcus granulosus* in the intestines of a dog during different seasons of the the year. Each dog was experimentally infected with 25,000 viable protoscolices.

No of Dog	Date of infection	Time of occurrence of eggs and proglotids in the faeces after infection.	Time of necropsy of dog after infection	Number of *Echinococcus* found	Mean strobila length (mm)	Mean length of last proglotid (mm)	Morphology of uterus in last segment
Summer							
1	10.07	33	35	21978	2.045	0.970	Filled with mature eggs
2	10.07	31	35	18317	2.538	1.410	
3 ■	10.07	32	35	19532	2.126	1.105	
4	10.07	35	35	18491	3.000	1.830	
Mean				19579.5	2.427	1.329	
Autumn 5							
6 7 8							
Mean	20.09	44	45	11910	4.050	2.110	Filled with mature eggs
	20.09	41	45	13427	4.895	2.950	
	20.09	39	45	10925	5.394	3.018	
	20.09	43	45	11435	5.203	2.946	
				11924.2	4.885	2.756	
Winter							
9	5.12	88	97	9356	4.253	2.046	Filled with mature eggs
10	5.12	83	97	1087 11547	4.036	2.018	
11	5.12	86	97	9864	3.534	1.696	
12	5.12	91	97	10409.5	3.820	2.010	
Mean					3.911	1.943	

CONTROL OF ECHINOCOCCOSIS

Prophylactic deworming of dogs is important for the protection of the health of people and agricultural animals. The aim of prophylactic therapy is to ensure dogs are treated before the infection becomes patent and thus minimise the potential for transmission. In areas where there are high levels of infection in animals, we believe it is necessary to treat farms dogs with anthelmintics 8 times per year. Of particular importance are treatments in late winter in February or early March; treatments in late April or early May; treatments in mid summer in June-July; and a fourth treatment when animals ar$ collected in the autumn.

To undertake the prophylactic treatment of dogs, a platform of 10x20m is necessary, where it is dry and there is no vegetation, preferably on a concrete surface. Treatment using

such a place can be undertaken on 10 dogs at a time. The anthelmintic arecoline is administered orally, and the process is considered complete when the dogs have defecated 3 times. All faeces and intestinal materials, together with any cestodes that have been passed are collected and burnt. The platform is disinfected with 10% chlorite solution. Used gowns, gloves and instruments are disinfected by boiling. Dogs that have been treated are recorded with their owners' names. This should be done 4 times per year. After each prophylactic treatment, owners are given anthelmintics to administer 30-40 days later. This treatment should not be hazardous as any parasites the dogs may have will be in the prepaptent period. Thus, this treatment administered by owners is also given 4 times per year. This scheme of anthelmintic treatment should be undertaken where there are the most intensive populations of dogs. We have also developed a two-stage process for the use of arecoline. A dose of 5mg/kg of arecoline is administered orally in a 0.1% solution. Any dogs that are seen to be infected with cestodes at the subsequent purge are given a second treatment. Using this method, we believe we can eliminate 100% of mature parasite infections and approximately 96% of immature parasites.

A modem method of administration of anthelmintics is the use of medicated feed for dogs. A maximum concentration of 10% anthelmintic is given in a product containing 70% wheat flour, 10% sugar, 8% milk, and 2% water. All components are carefully mixed and made into a bait, dried and stored in the dark for up to 1 year.

It is important that anthelmintic preparations are cheap and effective. Bunamidine hydrochloride at a dose of25-50 mg /kg is reported to be 100% effective against *E. granulosus* 90 days after infection. In a trial involving 25 dogs we found that parasites were completely eliminated in 24 dogs after 2 doses of bunamidine, and the overall parasite burden was reduced by 99%. We have also found that bunamidine has a good prophylactic effect when mixed with food granules and at doses of up to 120 mg/kg does not appear to have side effects in dogs.

Geksohlorofen at a rate of 15mg/kg has an efficacy of 78.5% against immature *E. granulosus,* rising to 100% with a second dose. At 50mg/kg the efficacy rises to 100% against both mature and immature parasites. The preparation cetovex (Rhone-Poulonc) has good prophylactic effect with a dose rate of 25 mg/kg being 97.4-99.4 % effective. We have also investigated the efficacy of praziquantal as an injection, tablet and in medicated granules. We have found that at the dose of 2.5-5.0 mg/kg the preparation is 100% effective against juvenile and adult stages of *E. granulosus* in dogs. We have also developed a new anthelmintic based on copper salts (cestan) in a fodder granule formulation, which is effective against the immature and mature forms of *E. granulosus* at 150mg/kg. The development of this product in particular means that we can avoid the importation of expensive and potentially toxic preparations from foreign countries.

One problem with the oral administration of anthelmintics is the possibility that the preparation is degraded by the acidic conditions in the stomach before it reaches the small intestine. Because of this we have developed an injectable anthelmintic preparation. This consists of 5% febantal, 10% fenasala and 3% mebendazole. This preparation at a dose of 25-50mg/kg given intramuscularly 25 days after experimental infection has an efficacy of between 74.4 % and 100.0 %. Mebendazole on its own appears to be 100% effective when given as a 3% injectable solution at a dose of 50.0 mg/kg. '

A very important aspect of the control of echinococcosis is the proper disposal of corpses of agricultural animals. We recommend that such animals are placed in secure

concrete tanks and incinerated wherever possible. Alternatively corpses treated with 10%/ formulin or 7% chlorite render cysts non infectious to dogs after 7-10 days. J

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