

Review article

The use of urban sewage sludge on pastures: the cysticercosis threat

Jacques CABARET^{a*}, Stanny GEERTS^b, Marilyn MADELINE^c,
Céline BALLANDONNE^c, Dominique BARBIER^c

^aPAP, INRA, 37380 Nouzilly, France

^bPrince Leopold Institute of Tropical Medicine, 155 Nationalestraat, 2000 Antwerpen, Belgium

^cFaculty of Pharmacy, Parasitology Laboratory, Bvd Becquerel, 14032 Caen, France

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Abstract – Urban sewage production is increasing and its agronomical use as a fertiliser has been advocated. Considerable defiance is prevalent in consumers and among farmers on the use of such fertilisers due to unknown pathological or environmental risks. The aim of the present review was to consider which pathological risk is major. Cysticercosis due to *Taenia saginata* appears to be one of the major pathological threats when sewage sludge is used to fertilise cattle pastures in temperate areas. The situation is different in Africa (*Taenia solium* and *T. saginata* are both highly prevalent) and Asia (*Taenia saginata*-like are prevalent). The processing of sludge and the delay between its application onto a pasture and grazing are probably major risk factors. Little data are available on the influence of processing, delay between processing and the use of sludge on the pathogenic risk. Producers and consumers will be more confident on the use of sludge if objective data are gained on risk. Most of the cases of cysticercosis (North America, United-Kingdom, Germany or Denmark) are related to poor human hygiene or accidental overflowing of sewage plants onto pastures. The standard application of sludge on pastures is apparently at low risk. This low risk does not mean that surveillance should cease since outbreaks of cysticercosis have been reported. Future investigations should concentrate on the most sustainable means of reducing risk (length of storage before use, composting, other treatments).

urban sludge / parasite / cysticercosis / cattle / human

Résumé – L'utilisation des boues d'épuration urbaine sur les pâturages : le risque de cysticercose. La production de boues urbaines résiduelles est en augmentation. Leur emploi agronomique comme fertilisant a été proposé. Une méfiance profonde des consommateurs ou des exploitants agricoles quant à l'emploi des boues urbaines en tant que fertilisants est réelle, qu'il s'agisse de risque pathologique ou environnemental. Le but de cette synthèse est de déterminer quel est le risque pathologique majeur. L'infestation par *Taenia saginata* semble être le risque le plus important en relation avec l'application

*Correspondence and reprints

Tel.: (33) 2 47427768; fax: (33) 2 47427774; e-mail: cabaret@tours.inra.fr

de boues d'épandages sur les pâturages bovins dans les régions tempérées. La situation est différente en Afrique (*Taenia solium* et *T. saginata* sont tous les deux très prévalents) et en Asie (les *Taenia* ressemblant à *T. saginata* ont un spectre d'hôtes herbivores assez large). Les processus de fabrication des boues et le délai entre le dépôt sur pâture et l'accès à la pâture sont sans doute des facteurs essentiels du risque. De rares données sont disponibles quant au délai et au traitement pour ce qui concerne leur pouvoir pathogène. Elles devraient être développées afin d'améliorer la confiance entre producteurs et consommateurs, sur des bases objectives. Le risque lié à l'épandage est faible comme le montrent des études aux USA, en Grande-Bretagne, Allemagne ou Danemark. La principale cause de risque repose sur un faible niveau d'hygiène humaine ou sur des mauvaises gestions des flux par les stations d'épuration qui déverseront sur des pâturages accidentellement des boues non traitées. Bien que le risque soit faible, la surveillance doit être maintenue car des épidémies locales ont été recensées. Les voies de recherches devraient être centrées sur les moyens durables de réduction du risque, à savoir, l'augmentation des durées de stockage des boues, leur compostage, ou d'autres traitements.

boue urbaine / parasite / cysticerose / bovin / humain

Table of contents

1. Introduction	577
2. Sludge: production, processing and uses	578
2.1. Production of urban sewage sludge	578
2.2. Sludge processing	578
2.3. Use of urban sewage sludge	579
3. Parasitic risks of using sewage sludge on pastures: an overview	580
4. Taeniasis/cysticercosis	583
4.1. <i>Taenia saginata</i> and <i>T. saginata</i> -like tapeworms	583
4.2. Human taeniasis	583
4.2.1. Prevalence and geographic distribution	583
4.2.2. Diagnostics and treatment	584
4.3. Animal cysticercosis	584
4.3.1. The intermediary hosts of <i>T. Saginata</i>	584
4.3.2. Prevalence and diagnosis in cattle	587
4.3.3. Sanitation and economic losses	587
4.3.4. Temporal and geographic distribution	587
4.3.5. Relationship between cattle and human infections.	588
5. Taeniids in urban sludge	588
5.1. Diagnostics	588
5.1.1. Identification	588
5.1.2. Estimation of egg numbers in sludge	589
5.1.3. Viability of eggs	589
5.2. Prevalence and intensity	590
6. Urban sludge and infection of herbivores with cysticercosis	590
6.1. Fertilisation of pastures using sludge	590
6.2. Critical review of cattle cysticercosis in relation to sludge fertilisation	591
6.2.1. The intensive use of sludge: the American examples.	591
6.2.2. The limited use of sludge: the European examples	592
7. Conclusions	593

1. INTRODUCTION

The passing of Clean Water Acts in developed countries in the 1970's has created a distinct quality standard that all waste water must meet through a pretreatment process. Sludge is an inevitable end product of modern wastewater treatment. The concentration of people in large towns has favoured the production of sludge on sites where no agricultural or forestry use can really be found: the sludges should then be exported to other sites where they may be received by populations and farmers as a kind of landfill disposal. Over the past 25 years, restrictions have been placed on certain sludge disposal practices (for example, ocean dumping and landfill disposals), causing public waste water treatment utilities to view the agricultural or forestry use of sludge as an increasingly cost-effective alternative. Sludge is not used in forestry in Europe, and its use has remained very limited in other parts of the world, except in the Pacific Northwestern USA since the 1970's. Agricultural land application of municipal waste water and sludge has been practiced for its beneficial effects and for disposal purposes since the advent of modern waste water management about 150 years ago. With the promotion of agricultural use for sludge, there has been increased public scrutiny of the potential health and environmental consequences of these reuse practices. There is a debate over whether recycling of sludges through land application as allowed under current regulations is protective enough, and whether it provides for a long term sustainable practice [52]. In France, the two major agricultural syndicates have not considered the current regulations protective enough, even though in the 30 years since the use of sludge, no major accident has been recorded [22]. Little data on sustainability are available and the data mainly concern heavy metals. Experiments at a number of long-term sludge treated field sites in the

United Kingdom, Germany, and Sweden, where metals have accumulated and persisted for decades, have shown that the microbial function in the soil is adversely affected by heavy metal concentrations that are not necessarily toxic to crops [71]. The use of sludge in agriculture has scientific and technical implications which are not met in their totality. In addition, this has given rise in some cases to the reluctance of farmers to use sludge as fertilisers, defiance of consumers and hence the ban of crops obtained on sludge fertilised farms by several multinational food producing or processing companies [9].

The concerns of using sludge as a fertiliser is not only for crops but also for food animals grazed or fed with fodder obtained on sludge fertilised areas. Animals that graze on land to which sludge has been applied to the surface will ingest sludge along with plants and some soil: grazing cattle may ingest 1–18% of their dry-matter intake as soil and sheep may ingest as much as 30% depending upon the management and supply of grass [34, 96]). Animals may then ingest toxins and pathogens. Sludges contain pollutants as well as a high concentration of pathogenic viruses, bacteria and parasites [35]. Most human pathogens which are found in raw sewage are concentrated in sludge. The levels and type of pathogens are dependent in part on the health of the human population contributing to the sewage plant and are affected by the type of sludge treatment. Some pathogens found in the sludges are zoonotic and should remain under control [90]). Infections with the helminths *Taenia saginata* in cattle or *Taenia solium* in pigs are one of the primary human zoonotic diseases associated with food. These helminths rely 100% on foodborn transmission (as Trichinellosis, Anisakiosis) in contrast with protozoan diseases (*Giardia lamblia*: 10%, *Cryptosporidium parvum* genotype 2: 10%, *Toxoplasma gondi*: 50%). *Taenia* eggs are not infrequent in untreated sewage sludge [67]).

The uncontrolled use of sludge to fertilise cattle pastures might then increase cysticercosis in cattle and consequently taeniasis in man consuming the meat of these infected cattle. In this review, we will concentrate on cysticercosis which represents the major parasitic problem linked to the use of sewage.

We will first characterise the production and uses of sludge in order to evaluate the potential risk of transmission of parasites on land fertilised by sludge. Secondly, we will give an overview of the potential parasitic risks using sludge on pastures. Thirdly, we will describe and estimate the available tools to diagnose taeniasis and cysticercosis in order to assess accurate prevalences of the diseases. Fourthly, we will present the diagnostic tools and derived prevalences of *Taenia* eggs in sludge. In the last paragraph, we will relate the presence of *Taenia* eggs to the infection of grazing ruminants. We will finally evaluate the pro and cons of utilising sludge in relation to the cysticercosis threat.

2. SLUDGE: PRODUCTION, PROCESSING AND USES

2.1. Production of urban sewage sludge

Most of the data presented are from Duvaud et al. [24] for Europe and Smith [92] for the USA. In the USA, the term of biosolids is used instead of sludge, and corresponds to municipal products that can safely be used on land application, and denotes municipal waste materials of the highest quality derived from waste water treatment. Production of sludge depends on the human population size, the population served by sewers and it increases as higher levels of wastewater treatment are introduced. In 1998, the production of sewage per capita ranged from 2.5 in Portugal to 37.7 kg dry matter (DM) in Denmark. We calculated that it was significantly correlated ($p = 0.04$) to the Gross National Prod-

uct (ranging from 8 900 \$ in Portugal to 28 500 \$ in Denmark, 1994 data for GNP): the Spearman rank correlation coefficient was 0.47 for 19 countries (Europe, Australia, Canada and the USA). The lower figures found in countries having a lower GNP are probably due to the fact that the population does not have access to the sewage system and thus a part of the produced sludge was not taken into account. In countries such as France that have intermediary amounts of sludge production (15.2 kg DM/inhabitant), 30% of the rural population have not access to the sewage system. Production of sludge is expected to increase in most developed countries attaining the highest values recorded in Denmark or the USA.

2.2. Sludge processing

Sewage sludge originates mainly from the sedimentation of the organic matter of waste water which is settled down in specifically designed basins of sewage treatment plants. Organic matter is chiefly composed of human excreta; it is easily fermentable and must undergo stabilisation before any kind of utilisation may be considered. The stabilisation procedures can be summed up as follows: drying (air or heat), chemical treatments, aerobic stabilisation, anaerobic stabilisation, or composting.

The pathogen reduction procedures can be divided into biological and nonbiological processes. The most used sanitation treatments are the following [23]:

- Non-biological processes: lime sanitation, air and heat drying, heat treatment (pasteurisation [55]);
- Biological processes: sludge lagooning, anaerobic digestion, mesophilic and thermophilic aerobic digestion (with or without subsequent anaerobic digestion), composting.

These processes are of the utmost importance for the infectivity of sludge. Their efficacies on *Taenia* eggs, which is one of the most resistant pathogens found in

sludge, are reported in Table I. The non-biological processing is not always as efficient as expected or has agronomical limitations (lime sanitation), has important financial costs and therefore is unavailable for small communities, and finally is not environment friendly. Biological processing necessitates large investments (anaerobic digestion for example) and is not always available for small communities. One simple factor, the decreasing infectivity of pathogens over time, has not yet been fully investigated and should be one avenue for future applied research. The efficacy of sludge processing on pathogen infectivity should be monitored on the most resistant pathogens; the sentinel chamber system (based on parasites within a chamber and exposed to sludge processing) is one of the possible systems for control (E. Fogarty, personal communication, 2001).

2.3. Use of urban sewage sludge

Currently a part of urban sludge is applied to the land for several beneficial purposes: vegetation for building up of

landscapes on highways and other sites (31% of the sludge) or for skiing tracks, compost making (24% in the Netherlands, highest value in Europe), and agricultural use on crops or pastures. Land fill is still frequent (90% in Greece and 81% in Italy), and dumping in the sea remains in a few European countries (Portugal: 58% in the sea and other waters; Ireland: 36% in the sea; United-Kingdom: 25% in the sea) [24]. Incineration ranges from 0 (Belgian Wallonia, Finland, Greece, Luxembourg, Portugal or Sweden) to 40% (Switzerland) or even 80% in Quebec, and we found that the main predictor of incineration use was the GNP (Spearman rank correlation of 19 countries = 0.50, $p = 0.03$). The percentage of sludge applied to agricultural land varies strongly: 99% for the Sydney Area in Australia to 4% in The Netherlands. This percentage is correlated to the amount of sludge produced per inhabitant (SPI). By using a different analysis (distance weighted least square, which is similar to successive local regressions), in Figure 1 we show that SPI and GNP (Gross National Product) are related to the percentage of sludge applied

Table I. Processing of the sludges and sanitation efficacy on *Taenia taeniaeformis* (Tt) or *Taenia saginata* (Ts).

Process	Sanitation of <i>Taenia</i> sp. (% efficacy)	Reference
Anaerobic digestion 35 °C 5–28 days	100 (Tt)	Olsen and Nansen, 1990 [76]
Anaerobic digestion	100 (Ts)	Pike, 1986 [78]
Lime treatment	100	
Lagooning 24 days	100	
Anaerobic digestion 35 °C 15 days	100 (Ts)	Morris et al., 1986 [73]*
Anaerobic digestion 35 °C 30 days	99.5	
Anaerobic digestion	99.9	
35 °C 10 days and lagooning 24 days		
Lime treatment	95	
Aerobic digestion at external temperature 16 days	83.4	
Aerobic digestion 50 °C 6 days	99.9	
Pasteurisation 60 °C 3 hours	99.1	

* Based on naive calf infection.

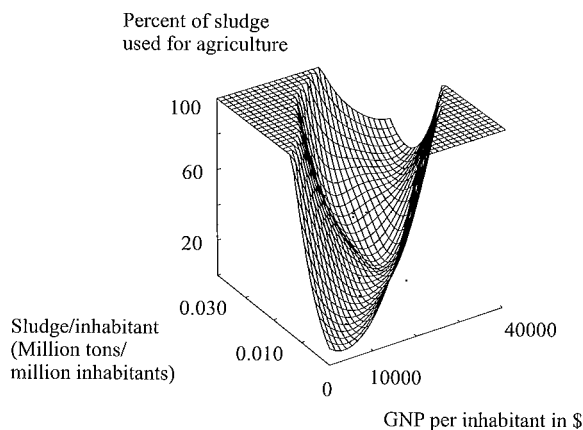


Figure 1. Relationship between the percentage of sewage sludge applied to agricultural land and the production of sludge per inhabitant and Gross National Product (GNP) per inhabitant in 21 countries.

on agricultural land: when SPI and GNP have low values, nearly no sludge was applied, whereas with higher values of SPI or GNP, the percentage of sludge applied on land increased. In the first situation, a low production of sludge was recorded and non-sustainable but inexpensive solutions were used (land fill or sea dumping). In the second situation, high production of sludge was associated with a higher concern for safety (incineration of sludge) and a sustainable (application on land) use of sludge. The total nitrogen input from sludge remains low (0 to 3%) compared with the inputs from animal origin (slurry, manure, etc.) or synthetic fertiliser. The situation was similar for phosphorus: sludge represented only 0 to 10% of the P input. A small proportion of sludge was used to fertilise pastures, although it could exceptionally reach 50% in Switzerland. This limited utilisation could be due to the reluctance of farmers or sewage plant heads, regarding the presence of pathogens in sludge.

3. PARASITIC RISKS OF USING SEWAGE SLUDGE ON PASTURES: AN OVERVIEW

A recent review of the literature on these risks is available [35]. The pathogens found in the 27 occurrences of sewage sludge fer-

tilisation are mostly *Salmonella* sp. (15 cases) and *T. saginata* (7 cases). We investigated which parasites are recovered in the sewage sludge from the CAB Abstracts database (1973–2001) and 160 references were found, but in fact few gave relative frequencies of infection with parasites. The data from both sources are presented in Table II. There is a large diversity in the hierarchy of proportions of the helminth genera, even in the same country (see data from France or Morocco for examples). The proportions are highly dependent on the human population on the site (an increase in *Ascaris* eggs was found in a community with many migrants in Norway [5]) and the sewage organisation (separated or not from other effluents), which explains the presence of *Toxascaris* and *Toxocara* from dogs and cats of non-domestic origin, or *Moniezia* from sheep of slaughterhouse origin). The helminth fauna of the human population are the main constituents of sludge helminths. In Marrakech, human infection can be arranged in the order of decreasing prevalence: *Ascaris* >> *Hymenolepis* > *Taenia* = *Trichuris*. In sludge, the order is somewhat comparable: *Ascaris* > *Trichuris* = *Hymenolepis* > *Taenia*. Surprisingly, this correspondence between human infection and parasites recovered in sludge is not so similar in several European countries where *Ascaris*

Table II. Parasites found in sewage sludge in several countries (interpreted from data extracted from the CAB Abstracts database 1973–2001, Furet et al. 1999 [35] and Khallaayoune, 1996 [60]).

Country	Helminths arranged in decreasing order of frequency*	Reference
Argentina		
Patagonia (4 sites)	<i>Hymenolepis</i> > <i>Ascaris</i> > <i>Trichuris</i> > <i>Toxocara</i>	Semenas et al., 1999 [87]
Bahrain		
	<i>Ascaris</i> > <i>Hymenolepis</i>	Amin, 1988 [1]
Brazil		
Parana (Curitiba, 22 samples)	<i>Ascaris</i> > <i>Hymenolepis</i> > <i>Trichuris</i> > <i>Toxocara</i> > <i>Taenia</i>	Thomaz-Soccol et al., 1997 [95]
France		
Caen (21 samples)	<i>Trichuris</i> > <i>Taenia</i> > <i>Ascaris</i> > <i>Toxocara</i>	Barbier et al., 1989 [3]
Caen (6 samples)	<i>Taenia</i> > <i>Trichuris</i> >> <i>Toxocara</i> > <i>Ascaris</i>	Madeline et al., 2001 [68]
Falaise	<i>Taenia</i> > <i>Toxocara</i>	Madeline et al., 2001 [68]
Nancy (several sites)	<i>Ascaris</i> > <i>Trichuris</i> > <i>Hymenolepis</i> = <i>Toxocara</i> > <i>Taenia</i>	Collomb et al., 1983 [15]
Nancy (1 site)	<i>Trichuris</i>	Own unpublished data 2001
Reims (19 samples)	<i>Ascaris</i> > <i>Toxocara</i> = <i>Taenia</i> > <i>Trichuris</i>	Bouchet and Léger, 1986 [10]
Champagne (14 samples)	<i>Trichuris</i> > <i>Ascaris</i> > <i>Toxocara</i>	
Savoie (38 samples in several sites)	<i>Taenia</i> = <i>Ascaris</i>	Gauthier et al., 1991 [40]
Tours (4 sites)	<i>Taenia</i> = <i>Trichuris</i> >> <i>Ascaris</i> > <i>Hymenolepis</i>	Own unpublished data, 2001
Valenton (1 sample)	<i>Trichuris</i> > <i>Taenia</i> > <i>Ascaris</i> = <i>Toxocara</i>	Madeline et al., 2001 [68]
Morocco		
Agadir	<i>Trichuris</i> >> <i>Ascaris</i> > <i>Hymenolepis</i> > <i>Taenia</i>	Khallaayoune, 1996 [60]
Beni-Mellal	<i>Hymenolepis</i> >> <i>Ascaris</i> > <i>Trichuris</i> > <i>Toxocara</i>	Khallaayoune, 1996 [60]

Table II (Continued).

Country	Helminths arranged in decreasing order of frequency*	Reference
Marrakech	<i>Ascaris</i> > <i>Hymenolepis</i> = <i>Trichuris</i> > <i>Taenia</i>	Khallaayoune, 1996 [60]
	<i>Ascaris</i> > <i>Trichuris</i> > <i>Toxocara</i> , <i>Hymenolepis</i> , <i>Taenia</i> , <i>Moniezia</i>	Bouhoum and Schwartzbrod, 1989 [11]
Ouarzazate	<i>Hymenolepis</i> > <i>Ascaris</i> > <i>Moniezia</i> > <i>Trichuris</i>	Khallaayoune, 1996 [60]
Norway		
(1 site)	<i>Ascaris</i> > <i>Trichuris</i>	Bergstrom, 1981 [5]
Switzerland		
(11 sites)	<i>Trichuris</i> > <i>Ascaris</i>	Eckert and Birbaum, 1983 [25]
Tchekia and Slovakia		
(72 samples, Slovakia)	<i>Ascaris</i> >> <i>Toxocara</i> > <i>Trichuris</i> > <i>Taenia</i>	Plachy and Juris, 1994 [79]
(3 sites in Bohemia)	<i>Ascaris</i> > <i>Toxocara</i> > <i>Trichuris</i> > <i>Hymenolepis</i> > <i>Toxascaris</i>	Horak, 1992 [54]
United-Kingdom		
East of England (7 sites)	<i>Ascaris</i> > <i>Trichuris</i> > <i>Taenia</i>	Watson et al., 1983 [100]
Ukraine		
(1 site)	<i>Ascaris</i> > <i>Trichuris</i>	Khizhnyak and Romanenko, 1975 [61]
USA		
(several sites in Illinois)	<i>Ascaris</i> > <i>Toxocara</i> > <i>Toxascaris</i> > <i>Trichuris</i> > <i>Hymenolepis</i>	Arther et al., 1981 [2]

* The sign >> means that the prevalence or intensity of one helminth infection is at least double that of the next one and an equal sign means that no information was available to arrange frequency or intensity.

** The generic names do not always correspond to the authors listing: we grouped *Trichuris* and *Capillaria*, and *Hymenolepis diminuta* and *Hymenolepis nana*.

is very infrequent in humans but is largely recovered in sludge; *Taenia* is not uncommon but is not always found in sludge. It is not yet clear if these discrepancies are real, or artefactual and due to identification difficulties (*Ascaris*) or technical problems recovering the eggs (*Taenia*). The identification

difficulties will be discussed in the paragraph on the identification of eggs in sludge. All parasitic risks are not described as far as ruminants are concerned: outbreaks of *Sarcocystis* have been recorded. Concerning animal and human health, *Taenia saginata* is probably one of the most

important problems, since it has been found in nearly all the sludges investigated under temperate or tropical climates.

4. TAENIASIS/CYSTICERCOSIS

4.1. *Taenia saginata* and *T. saginata*-like tapeworms

T. saginata presents much variability. In most parts of the world cystercerci of *T. saginata* are present in the muscles of cattle. On the contrary to common belief, true predilection sites do not really exist, except for the heart, where the highest number of cystercerci per kg of muscle is found [41]. In the extreme north of Russia, where bovines are scarce and people are used to eating raw reindeer meat, *T. saginata* uses the reindeer as an intermediate host. The main localisation of cysticerci is at the surface of the brain [62]. In Russia two strains have been evidenced, the northern one in Reindeer and the southern strain in cattle. The northern strain (from the Yamal Peninsula) could also infect calves with a lower success than reindeer and cysticerci have been located in the elective sites of cattle but not in the brain. The infection of reindeer remains low in nature with 2 out of 413 [62] and 0 out of 346 [63] being infected as with human infection. *T. saginata* has been considered as the dominant species in the Asian-Pacific region, despite the fact that in Taiwan, Korea, Indonesia and the Philippines people rarely eat beef. All infected Taiwan aborigines have the habit of eating raw meat and viscera of animals. Among these infected aborigines, 73% eat wild boars, 66% flying squirrel, 65% wild goats, 56% muntjacs, 49% wild rats, 46% monkeys, 38% hares, and no domestic animals [28]. A number of features distinguish Asian *Taenia* from the other human taeniids. They can infect cattle, goats, monkeys, wild boars but pigs are the dominant intermediate hosts and the cysticerci are usually found in the liver. Early molecular

studies and gross morphology suggest similarity with *T. saginata*. Although Asian *Taenia* can be recognised using Restricted Fragment Length Polymorphism on the cytochrome c oxidase I gene (Msp 1 recognition site) and is a genetically distinct entity, its very high similarity with *T. saginata* qualifies the Asian *Taenia* as a subspecies or a strain of *T. saginata* [72]. The Asian *Taenia* is not the only *Taenia* in Asia: all isolates studied from Taiwan (15), Korea (3), Malaysia (2), China (2), the Philippines (3) and Indonesia (4) belonged to the Asian *Taenia* strain whereas this strain was not found in 10 Thai isolates. *T. saginata* (the ordinary or variant strain) and *T. solium* are thus present all throughout Asia and in this review we will include the Asian strain with *T. saginata*. Additional studies on the genetic structure of *T. saginata* populations are necessary in order to distinguish what is a strain and what is a phenotypic modification in unusual hosts.

4.2. Human taeniasis

4.2.1. Prevalence and geographic distribution

The diagnostic in humans, and hence prevalence, depends much on the technique employed: thus perianal swabbing is more effective (80 to 88.9%) in the diagnosis of *Taenia saginata* infection in man than either faecal examinations (40 to 44.4%) or verbal evidence (21 to 23.3%) [63]. In Europe, *T. saginata* is the main cestode found: there were 634 case reports on *T. saginata* whereas there were only 6 on *T. solium* (in Poland) over the last 25 years. The situation is extremely different in other parts of the world. Latin America and some parts of Africa or Asia have a high prevalence of infection [66]. Neurocysticercosis cases due to *T. solium* may reach 400 000 people in Latin America and be endemic in the highlands of Peru with a prevalence reaching 14% [6]. We will concentrate in this review on *T. saginata*, but the use of raw sewage

sludge in these endemic areas with *T. solium* infection could constitute a real danger. Data on prevalence are presented in a review by Pawlowsky and Schultz [77]. They reported detailed prevalences in the previous USSR, indicating that overall prevalence was 0.6% in 1950, 0.3% in 1960 and 0.08% in 1966. An endemic foci was noted in the Caucasian area (Dagestan, Azerbaidjan, Armenia, Uzbekistan, and Kazakhstan) with prevalences always higher than 10%. Another endemic focus was recorded in part of the former Yugoslavia (Bosnia and Montenegro). They also reported another endemic foci in Africa (Abyssinia, Sudan, Senegal, Kenya, Congo and South Africa). These data need to be updated and we consulted the helminth database from the Commonwealth Agricultural Bureau (1973–June 2000) and found 1786 papers on *Taenia saginata* during this period. The reported results from the last 25 years are presented in Table III. *T. saginata* is a cosmopolitan parasite but the prevalences are relatively low (less than 10%) in other sites than Dagestan where 36% of the people are infected. The prevalences are highly variable within a country (see Turkey from 1.8% to 10% or France from less than 0.01 to 0.3%) and between countries. This variability in prevalence is due to hygienic habits (human defecations available or not to cattle), quality of meat inspection (decreasing the availability of contaminated meat to man) and culinary habits (the use of raw or poorly cooked preparations will permit the survival of infective cysticercus in meat). The local distribution of disease is very likely patchy in relation to these local environments and cultural behaviours. In sites where *T. solium* is also present (some parts of Africa for example), the prevalence of *T. saginata* can be slightly inflated due to misdiagnosing. The prevalences in man are not well established, due to selective sampling (a limited area, a category of workers, human populations from hospitals) and are only indicative.

4.2.2. Diagnostics and treatment

Differential diagnostics of *T. saginata* and *T. solium* on eggs is not really easy. Each egg consists of an outer shell, chorionic membrane, and a thick and striated embryophore. The morphology of *T. saginata* and *T. solium* embryophores does not allow identification but Ziehl-Nelsen staining is positive in *T. saginata* and negative in *T. solium* [75], although this has not been confirmed by other authors [81]. Currently, however, Polymerase Chain Reaction tests, have been developed, which allow differential diagnosis of both taeniids, on the basis of the eggs [47]. The characteristics of both worms are as follows:

T. saginata: proglottides are 15–20 mm/6–7 mm and are located on the surface of faeces and can be eliminated between defecations. The longitudinal uterus has 20 to 30 lateral dichotomous branches. Eggs are found at the anus margin and perianal swabbings are effective.

T. solium: proglottides are within the faeces. The uterus has 7 to 13 lateral branches. Eggs are found in the faeces.

Niclosamide and praziquantel are used to treat taeniosis [84]. They are lytic for the parasite in the intestine but do not alter the viability of eggs which are eliminated in the faeces and are later found in sewage sludge. When *T. solium* infection is suspected, saline purgation is advocated.

4.3. Animal cysticercosis

4.3.1. The intermediary hosts of *T. saginata*

Cestode larvae are less specific than adult cestodes, and therefore the list of intermediary hosts is considerable. The main intermediary hosts are domestic cattle. We evaluated the possible role of other intermediary hosts of *T. saginata* on the CAB Abstracts database (1973–2000). Our index was built on 1752 references on *T. saginata*

Table III. Prevalence of human taeniasis (*Taenia saginata*) in different parts of the world as obtained from a review of published papers from 1973 to 2000 (CAB Abstracts database).

Continents and countries	Prevalence (%)
Europe	< 0.01–10
Bulgaria, France*, United-Kingdom	< 0.01
Belgium, France, Germany, Hungary, Italy, Montenegro, Poland, Spain, The Netherlands, Turkey*	0.01–2
Slovakia, Turkey	> 2–10
Americas	< 0.01–10
Brazil, Canada, Chili, USA	< 0.01
Argentina	< 0.01–2
Honduras	2–10
Africa	< 0.01–7
Cameroon, Egypt*, Ivory coast, Morocco, Sudan, Tunisia	0.01–2
Cameroon*, Egypt	> 2–10
Asia	0.02–36
India* (Uttar Pradesh), Iran, Pakistan	0.01–2
Afghanistan, Azerbaidjan, India (others), Malaysia	2–10
Dagestan	36

* Country appearing twice since two surveys gave different results.

as follows: reference in one host species infected/total number of references that is 1752/total number of references on the intermediary host species. The data are shown in Table IV. With our index, reindeer becomes a very important host, followed by cattle: this means that the index gives an indication of the preoccupation that investigators have for the host species as an intermediary host among other interests regarding the species. It does not include the Asian *Taenia* since the references did not always indicate whether this *Taenia* was *T. saginata*. Domestic cattle (*Bos taurus*: see Tabs. IV and V, *Bos indicus* [17], or buffaloes [18]) remains the dominant intermediate host. The role of sheep and goats as hosts

for *T. saginata* remains obscure. Goats were found to be naturally infected in Assam: 2.7% compared with 5.7% in cattle [82]. The experimental infection of sheep and goats was unsuccessful for Blazek and Schramlova [7] whereas Kozakiewicz [64] infected sheep and goats, with the structure and location of the cysticerci being different in unusual hosts [85]. Sheep and goats, even if they do not serve as very effective intermediary hosts, may suffer from infection and this should be considered if sewage sludge is used on sheep and goat pastures. The report of the lama being an intermediary host is to be taken cautiously since the cysticerci could have been confounded with *Sarcocystis* cysts (Schmidt E., personal

Table IV. Putative intermediate hosts of *Taenia saginata* as shown in the total 1752 references on that species, reported from 1973 to 2000 in the CAB Abstracts database.

Intermediate host species	Number of references for a species (all interests), <i>a</i>	Number of references of the species as intermediate host, <i>b</i>	Index of importance ($(b/1752/a) \times 10^7$)
Cattle	233931	814	19.9
Sheep	111324	81	4.2
Goats	32970	32	5.5
Reindeer	1693	11	37.1
Lama	1127	1	5.1
Chamois	449	0	0
Roe deer and <i>Dama dama</i>	4341	2	2.6
Red deer	2084	0	0
Lagomorphs	29108	33	6.5

Table V. Prevalence of bovine cysticercosis and incidence of human taeniasis in some European countries [86].

Country	Cysticercosis ^a Prevalence (%)	Taeniasis ^b incidence (%)	Year
Denmark	0.1–0.7	0.02	1990
Germany:			
former East	4.5–6.8	0.33–0.62	1993
former West	0.4–0.8	0.09	1985
Netherlands	1.8–2.2	0.14	1985
Belgium	0.03–0.2	0.26–0.46	1992
Spain	0.007–0.1	—	1999
Poland	0.24	1.64	1999
Italy	0.02–2.4	0.02–0.04	1999

^aBased on abattoir data.^bBased on sales figures of specific antiparasitic drugs in humans.

communication, 2001). The infection of lagomorphs remains unclear since other cestodes may infect them. The infection of roe-deer is also questionable since Boev [8] showed that the cysticerci found were not those of *T. saginata*. Unhooked cysticerci are found in Africa in antelopes: wildebeest, bush-buck [75], and in several species of gazelles. They cannot be attributed

with certainty to *T. saginata*. The role of wild ruminants in Africa has to be further studied. These considerations on wild mammals such as lagomorphs, roe-deer or red deer as intermediate hosts of *T. saginata*, although unresolved, are of considerable importance if sewage sludge is to be used for fertilising forests in temperate areas. The experimental infection of

Meriones unguiculatus (by injection: [101]) and sheep (oral route: [44]) did not prove very effective; however the influence of sewage sludge fertilisation on pastures grazed by rodents and small ruminants remains unclear and requires further investigation.

4.3.2. Prevalence and diagnosis in cattle

According to the official meat inspection figures, bovine cysticercosis is present at a low level in the countries of the European Union (Tab. V). Its prevalence varies between 0.007 and 6.8%. Much higher prevalence figures (> 20%) for bovine cysticercosis are found in several countries of East and Central Africa (Geerts, 1993 [42]). Detailed inspection of predilection sites (head and/or heart) have however, shown 5 to 50 times higher prevalence rates than what is found upon routine meat inspection [20, 41, 43]. Furthermore, Walther and Koske [98] and McCool [70] showed that in 49–51% of the lightly infected animals, cysticerci are not present in the so called predilection sites (heart, masseter, oesophagus, diaphragm and tongue). This implies that the current 'knife and eye' method is able to detect only the tip of the iceberg and that the majority of cysticercosis cases escapes at the slaughterhouse.

Therefore, several research groups have tried to develop serological tests in order to improve the detection of cattle infected with cysticercosis. Many different antibody detection tests have been described using homologous or heterologous (*Taenia crassiceps* or *Taenia hydatigena* metacestode) antigens [44, 86]. However, most of these tests are not reliable because they do not differentiate between animals harbouring live metacestodes and animals in which only degenerate metacestodes are present, which are no longer infective. The development of a monoclonal antibody based antigen detection ELISA has allowed to identify those cattle harbouring living cysts [13, 50]. This tech-

nique has been further improved by using monoclonal antibodies of the IgG isotype and by pretreatment of the serum by heat or trichloro-acetic acid in order to dissociate immune complexes. The specificity and sensitivity of the antigen detection ELISA are 98.7 and 92.3% (if the animals harbour more than 50 live cysticerci), respectively [59]. With this improved ELISA, 3.1% of the Belgian slaughtercattle were found to be positive whereas using the classical meat inspection cysticerci, only 0.26% were detected [21].

4.3.3. Sanitation and economic losses

In Europe, carcasses infected with cysticerci are usually frozen for 10 days at -10°C . Only the heavily infected carcasses are completely condemned. It has also been shown that low level gamma irradiation (0.3–0.6 kGy) is able to kill *T. saginata* cysticerci [46]. The economic loss due to bovine cysticercosis is caused by the cost of: 1. transport to an official freezing installation; 2. the freezing of the carcass; 3. a certain weight loss due to freezing (2–5%) and 4. a loss of the value of deepfrozen meat (as compared to fresh meat). These losses amount to 30 to 45% of the value of the carcasses, a figure which is also used by some insurance companies [41].

4.3.4. Temporal and geographic distribution

Data on the geographic distribution of *T. saginata* cysticercosis mostly originates from cattle meat inspection reports. They are grossly defective, since efforts on diagnostics are not identical from one site to another, and data are recorded over a large period of time. Our data are taken from the review of Pawlowsky and Schultz [77] and from the CAB Abstracts database 1973–2001 (more than 880 references). Clearly there are variations along time: prevalences decreased, increased or remained the same according to the countries. Prevalence

decreased from 4.6 to 0.2% in Azerbaijan (from 1960 to 1987), from 0.29 to 0.023% in Greece (before 1969 and after 1978), from 0.4 to 0.03% in UK (1963 to 1979), and in Hungary 0.28 to 0.06% (1956 to 1983). This decreasing trend is attributed to human treatments (Azerbaijan) or improved meat control (UK or Hungary). The prevalences remained stable in Denmark (approximately 1%) from 1918 to 1982 and in Italy (the region of Torino): 0.10 (1946–1955), 0.09 (1956–1965) and 0.12% (1966–1975). In other countries, prevalences increased: 0.7 to 1.3% in Czech Republic (1989 to 1998), 3.5 to 6.8% in the former German Democratic Republic (1974 to 1989), 0.4 to 1.2% in the former West Germany (1970 to 1990), and 0.34 to 1.6 and 3.1 in Praha (1945, 1955 and 1964). Prevalences may increase due to the importation of infected meat (Norway), or there may be a local increase of infection risk (Use of sewage sludge in German and Russian cases? Poor hygiene of farm workers in Russian cases?).

4.3.5. Relationship between cattle and human infections

We excluded the so-called Asian *Taenia* as well as the transmission by means of the reindeer since several human populations concerned are used to consuming raw meat and thus transmission is evidently more efficient. No general relationship has really been established so far since most studies do not include cysticercosis in cattle and human *T. saginata* infection surveys for each studied site. We selected 21 pairs of data corresponding to the simultaneous investigation of *T. saginata* larvae in cattle and adult worms in man in one region. They corresponded to data from Bulgaria, Caucasus, France, Greece, Italy, India, Nigeria, Poland, Slovakia, and the USA, collected from references from 1970 to the present. The Spearman coefficient of correlation was 0.81 ($p < 0.05$). The good relationship between the two host infections

indicates that careful attention must be paid to cattle infection and hence to cysticercosis storms that might be related to sewage sludge misuses.

5. TAENIIDS IN URBAN SLUDGE

5.1. Diagnostics

5.1.1. Identification

The helminth eggs in human and animal faeces have been largely described in textbooks. The qualitative characteristics of Trichurid eggs (*Trichuris* sp. or *Capillaria* sp.), Hymenolepids, Taeniids, and human or animal Ascarids, make these four categories easily discernable for a practiced helminthologist. They are not as obvious for a routine diagnostic laboratory irregularly handling sludge, as we found in a multicentric assay in France (our own unpublished data). Eighteen laboratories were involved and only 12 could deliver accurate results. This was due in part to a limited experience, but could also be related to the modification of eggs in sludge. The qualitative characteristics were not modified but size modification could have explained the misidentifications. For this reason, the helminth eggs were examined in digested sludge from 6 sites (the Normandy and Paris area in France) and they were either lime hygienised or not (own unpublished data). The treatment with lime or the origin of sludge did not significantly modify the size of the eggs. A few *Ascaris* eggs were found and the ranges of the length and width of the eggs were respectively 56–67 and 46–53 μm . The following key was established for the most frequently recovered eggs in sludge, based on 95% confidence intervals of the mean after resampling of the data (21 *Toxocara/Toxascaris*, 65 Trichurid, 102 Taeniid eggs):

(1) Subspherical eggs, 11 Small: 35–36 μm diameter Taeniid eggs, 12 Large:

79–84 μm diameter *Toxocara* and *Toxascaris*,

(2) Elongated eggs: 56–60 μm length and 28–30 μm width: Trichurid eggs.

The sizes of the eggs in sludge were not different from those recovered in the faeces; this was a significant help for the diagnostics. The individual size of the eggs of Taeniids ranged from 35 to 40 μm .

5.1.2. Estimation of egg numbers in sludge

A large number of methods has been proposed for detecting helminth eggs in waste water [37] or sludge (US EPA technique 1992, triple flotation technique [4] derived from [29]). The techniques are based either on sedimentation or flotation using a wide variety of flotation solutions (MgSO_4 , ZnSO_4 , and NaNO_3 at several concentrations: see [37] and most of them are satisfying for detecting nematode eggs. Several of these techniques were tested on sludge with Taeniid eggs [67]. The US EPA (density 1.2 of the zinc sulphate flotation solution), modified EPA recommended in France ($d = 1.30$), and the EPA using a $d = 1.38$ flotation solution were poorly effective in recovering of Taeniid eggs, respectively in 0, 1, and 2 sludge samples out of 10. The triple flotation technique yielded positive results in 6 of these samples. Most of the techniques have a low recovery efficiency and the most efficient triple flotation technique ranged from 19 to 48% for Taeniid eggs. Improvements are needed if one wants to exactly assess the risk due to sludge application on pastures, but triple flotation already warrants a sufficient sensitivity for epidemiological surveys.

5.1.3. Viability of eggs

The presence of a pathogen in sludge does not warrant its viability and infectivity. The identifications based on morphology or the polymerase chain reaction (PCR) do not give information on the

ability to develop into further stages. Viability is an important criterion and nematodes were chosen as indicators for parasitic helminths in France because they were the only helminths that could be easily cultivated in laboratory conditions. The class A pathogen requirements in the USA also base viability on the examination of nematode eggs using *Ascaris lumbricoides* var. *suum* eggs as the control: the results are available after 3 to 4 weeks of cultivation. More rapid procedures have been proposed (30 °C in deionised water) and with larval observation after 8, 10, 13 and 16 days for nematodes, *Capillaria* sp., *Toxocara canis*, *Ascaris suum*, and *Trichuris vulpis*, respectively [38]. In vitro hatching of *T. saginata* eggs is a rapid procedure (one to a few hours) that has been used widely, but is not always satisfactory although it has been improved [93] using digestive enzymes. Later, it was compared to *Taenia solium* with a technique using sodium hypochlorite, the hatching rate being increased with the latter [99]. The reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by mitochondrial enzymes to its blue formazan product is a widely used colorimetric assay method to determine the viability of cells in culture or of pollen and has been used for parasites. Kanazawa et al. [58] and Emery et al. [26] on the cestode *Echinococcus multilocularis* reported that living elements are coloured in blue and Kanazawa et al. [58] showed that they are also infective. This staining procedure then appears as a simple way to categorise living versus dead parasitic elements. Some caution must remain especially when using a complex media such as sludge: it was shown that plant catechols and flavonoids reduce MTT through iron ions [48]. A modified technique based on MTT staining has been tested on *T. saginata* eggs in sludge [68, 69]. This technique was derived from a technique used in MDH (malate dehydrogenase) isoenzyme studies [36]: the chemicals needed to ensure the reaction (not only MTT) are all

provided except the enzyme. When the *T. saginata* eggs do not have MDH activity (then they are considered dead), they do remain brown-yellow; otherwise they are stained in blue. The MTT technique gives useful information on the viability in anaerobic digested or lime hygienised sludge [68] but it should be tested in a large array of sludge types; the relationship between viability and infectivity should also be evaluated. Infectivity is fairly low [73, 76] and viability should then also be low, which was the case in our unpublished data using the MTT technique (from 0 to 50%).

5.2. Prevalence and intensity

Taenia is frequently recorded in sewage sludge (see Tab. III). No prevalence in any area can be established since controls on the presence of *Taenia* are highly dependent on techniques and no data collection and analysis has ever been performed. Using the same technique (triple flotation with zinc sulphate at 1.38 density) in 2001 in several sewage plants, we found (unpublished data) that *Taenia* was recovered irregularly in the French Touraine sludges that were not sanitised. Tours (a large town with over 200 000 inhabitants), Chateau-Renault (a smaller town with more than 8 000 inhabitants) were tested positive, whereas smaller towns such as Monnaie (over 2 000 inhabitants), Nouzilly (over 1 000 inhabitants), or the INRA research station sewage plant (less than 700 persons) were uninfected on at least two samplings in the Spring. The fact that the few small plants investigated did not harbour *Taenia* eggs might be due to the fact that prevalence in humans is fairly low on the average so that they may often be recorded as uninfected whereas larger towns have a higher chance to harbour infected humans and hence eggs in their sludge. When infected, intensity is a major concern when sludge is intended as fertilisers, since heavily contaminated materials will ensure better transmission. It was

found that 2.5 eggs were recovered per gramme of sludge (25% dry-matter) and the authors calculated that this might correspond to a 1% human infection [3] which is in the range of medium to high infection in western Europe (see Tabs. II and V).

6. URBAN SLUDGE AND INFECTION OF HERBIVORES WITH CYSTICERCOSIS

Sewage is an important means of spreading *T. saginata* infection between the human population and cattle as was observed long ago [77]. The use of sludge as a fertiliser is only one of the many possibilities.

6.1. Fertilisation of pastures using sludge

We investigated the importance of fertilisation with sludge and which helminths were associated with sludge in the CAB Abstracts Database (1973–August 2001). Fertilisation studies generated 89980 papers, sewage sludge 7320, and sewage sludge and fertilisation 1260 papers during that period. This indicates that sewage sludge plays a very small role in fertilisation. It plays an even more limited role in the fertilisation of pastures (only 23 references). The publication effort is not always a good reflect of disease importance and the above figures should be taken as indicative. These general considerations on the limited importance of sewage sludge as a fertiliser are locally found in many medium size towns like Tours in France. This town produced 13700 tons of sludge in 1997 and needed 685 ha/year for sludge spreading. Sludge is used as a fertiliser in the winter and autumn, and mainly concerns maize (over 40% of fertilisation); it is not used on pastures. The relative importance of sludge in the transmission of helminths can also be assessed by the proportion of references dedicated to one group of helminths in sludge in relation

to the total number of references reported for the same group of helminths. This proportion was 1.1 per thousand for nematodes and 2.0 per thousand in cestodes. It was 5.6, 0.3, 9.1, 3.2 per thousand, respectively for *Taenia*, *Hymenolepis*, *Ascaris* and *Trichuris*. Only *T. saginata* was investigated among Taeniids and no information was available on *T. solium*. Few data are available on the parasitological contamination of urban sludge and the parasitological risk was officially first taken into account in Switzerland in the Obsubst ordonnance [94] and secondly in the USA [97]. The use of sludge as a fertiliser was legally described later in France for example [33]: it can be spread on pastures three to six weeks before forage is cut or the pasture is grazed by animals, depending on the presence or absence of sanitisation of the sludge. The problem of the regulations is that helminth eggs are all grouped together as helminths or nematodes and cestodes: in Switzerland no helminth eggs should be recovered, in the USA the class A sludge should have a concentration lower than one egg per four grams of dry-matter, and the French regulations indicate that the number of viable helminth eggs should be lower than 3 per 10 grams of dry-matter. Parasitological contamination is the rule and has been extensively studied in 89 French urban sludges [39]. Forty-seven percent of sludge had helminth concentrations lower than 6 eggs/10 grams dry-matter, 38% had concentrations ranging from 6 to 24 eggs/10g, whereas the rest had concentrations over 24 eggs/10 g. The number of helminth eggs in sludge is a general indicator of contamination but does not allow risk assessment for a particular host. The risk may concern humans who manipulate the sludge, crops (many species of nematodes are plant parasites), or animals (cattle susceptible to *T. saginata* or other herbivores). The risk for the mans has mainly been evaluated for waste waters used for irrigation [12, 19, 30, 89] and concerns general pathology rather than one particular pathogen. Sinnecker

[89] in Germany noticed a higher infection in agricultural workers for *Ascaris* and *Trichuris trichiura* and Bouhoum et al. [12] in Morocco recorded a higher infection of children in the irrigated areas for the same parasites and did not find any difference in *T. saginata*, *Hymenolepis*, and *Enterobius*. The risk assessment in man should then be related to *Ascaris* or *T. trichiura*. The risk for crops should focus on fungi and nematodes specific to plants. The helminth risk for grazing animals are *T. solium* (grazing pigs) or *T. saginata* (cattle) infections. The presence of *T. saginata* in sludge in Europe and North America appears as one of the major concerns of using sludge as a fertiliser on cattle pastures and a specific risk for evaluation is really needed [14]. This concern is particularly true in areas having high records of cysticercosis (for example 22%, in the German Potsdam district); a direct correlation was noted between cattle infections and the areas fertilised by sewage [27].

6.2. Critical review of cattle cysticercosis in relation to sludge fertilisation

6.2.1. The intensive use of sludge: the American examples

Although the infection rates of cattle have been relatively low (less than 0.08% in 1967) in the USA, local outbreaks of cysticercosis have been recorded. These outbreaks are attributed tentatively to: leakage of raw sewage from sewage plants, contamination of feed or water by farm employees or application of sludge on pastures. Upon the post-mortem examination of 230 animals, Fertig and Dorn [31] found 37 infected from a single farm in Ohio. The farm had received applications of municipal sewage sludge (2% dry matter) for the last twenty years. Sewage sludge stabilised by aerobic digestion was applicated on the pastures. Sludges (0.31 to 2.04 ton/ha) were used on one to three occasions during the

grazing season. A municipal sewage treatment plant was adjacent to one corner of the farm, downstream from a 5 to 10 m wide creek that ran through the pastures; following the creek there was a sewer line in the pastures that terminated at the sewage plant and several manholes, covered with loose fitting tops. Cattle had access to these manholes. Control of the employees did not show *Taenia* infection. The infection of cattle was attributed to leakage of the raw sewage after a flood corresponding to heavy rainfall. The role of sludge application was discarded since the cysts could not have reached calcification by the time of the exposition of the cattle to the sludge fertilised pasture. Another outbreak was recorded two years later on the same farm and was associated with sludge applications which were repeated as frequently as six to seven times on the pasture all along the grazing season. Sludge was implicated in one outbreak in Virginia [49] whereas three other outbreaks were attributed to human faecal contamination of feed or water in Kansas and California [16, 91]. The Virginian outbreak was intense: 18 out of 40 steers were infected. For four years, grazing areas were fertilised with oxygen-activated sludge in a municipal plant which did not use any heating phases. Application of the sludge on pastures were done on a two month rotating basis from April to November each year. Cattle were returned to these pastures within two or three weeks of sludge application. The repeated applications of untreated sludge and a short period before the return of the cattle on fertilised pastures are apparently the reason for such outbreaks in cattle grazed on the pastures.

6.2.2. The limited use of sludge: the European examples

The influence of the application of sewage sludge was extensively studied in Scotland before 1990. Five outbreaks were observed in 1976–1979 and were traced to the application of sewage sludge to grazing

land [32]. Guidelines, issued since 1979 on the field application of sewage to ensure the control of pathogens, specify that livestock should not graze on pastures treated with undigested liquid sludge for six months, or digested liquid sludge for 3 weeks, or until 10 cm growth of herbage has occurred [52]. Digested sludge is defined as sludge produced either by a two-year storage of raw sludge or by aerobic digestion at 30 °C for 30 days. To really ensure that no viable *Taenia* eggs are present, a sixth month no grazing period is required. Under these conditions sludge application on pastures can no longer explain infection of cattle. In Scottish cattle, 1966 sera were observed: sera from cattle which had grazed on pastures to which sewage had been applied, were indistinguishable from uninfected animals [88]. During meat inspection, 264 cases of *Cysticercus bovis* were identified in cattle and then traced back to 218 farms of origin and the possible association with the application of sewage sludge was investigated. Only 16 cases came from farms using sewage sludge [80] and the use of sewage sludge was not directly implicated in the cattle cysticercosis. In another study [51], 4 farms using sludge application on pastures were compared to 5 farms not using sludge: the cattle Elisa test based diagnostic indicated that 1 and 0 farms were positive, respectively. This latter investigation indicates that even if sludge may play a role in cysticercosis infection, it remains limited as already described by Pike [78].

Another outbreak series occurred in Germany, in the region of Aachen [83]. In 1986, the average rate of complaints of cattle cysticercosis at the Aachen abattoir was 6.4% every month and even reached 15.2% which was very unusual (see prevalences in Tab. V). The infected cattle came from the urban and rural districts of Aachen. One hundred and twelve farms from the area were studied, only 38% of which were connected to the sewerage, 26% led their waste water into a one-tank or a three-tank system

and the rest distributed it in their dung-water or liquid manure tanks. Only a few farmers used sewage sludge as fertiliser. The exact cause of cysticercosis was difficult to assess in the infected farms since quite often, more than one risk factor could be demonstrated. The infection of workers on the farm was probably one of the most important causes as well as the use of contaminated water (irrigation of pastures or drinking water) due to the fact that some villages delivered their sludges, without treatment, into the water system.

A few records of cysticercosis outbreaks in the 1970's are given by Haugaard [53], the sources probably being the application of untreated sludge in one case. Later, an increasing number of cysticercosis outbreaks in cattle were reported in Denmark [74]. In 15 outbreaks, the infection sources likely responsible are sewage sludge deposited on pastures, flooded streams containing sewage effluents, and human excreta spread with animal slurry. The restrictive rules for the agricultural use of sewage sludge have been implemented in Denmark since 1984 and are not favourable to the transmission of cysticercosis: when raw sludge is directly deposited into the soil, such areas should not be used as pastures, for a period of one year. Ilsoe et al. [56] studied the possible sources of infection in farms presenting heavy cattle infection. Sewage sludge was not used as fertiliser on any of the farms. In six farms, septic tank sludge was applied on grass or feed, two had grazing sites near the sewage plants, and human defecation on the pasture was a problem on three farms. Poor hygiene or poor control of sewage plant overflowing were apparently the main sources of contamination as found with the German outbreaks. A case-control study in light-infected cattle versus uninfected cattle was then undertaken in Denmark [57, 65] and confirms in part this statement. The major risk factor identified is allowing the cattle to drink from streams carrying effluents from sewage treatment

plants (odds ratio: 3.6) and the other factors recorded in preceding reports (such as the application of sludge) appeared to be of minor importance. This does not mean that we should be overconfident on regulations concerning sludge application: the eggs of *T. saginata* can survive long periods on pastures. Ilsoe et al. [56] showed that a small proportion of eggs survived for 5.5 but not 8.5 months when deposited in May and for 5.5 months but not for 8.5 months when deposited in September.

7. CONCLUSIONS

The use of sludge for fertilising pastures is ecologically sustainable compared to other processes (incineration among others). It has some disadvantages, one of them being the putative introduction of pathogens. The seeding of *T. saginata* eggs from municipal sludge onto pastures is of limited risk if the sludge is properly digested, eventually sanitised, and if sludge application is conform to the rules imposed in most developed countries. Sanitation of sludge and proper use is the warrant of a negligible risk for cattle and human health.

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REFERENCES

- [1] Amin O.M., Pathogenic micro-organisms and helminths in sewage products, Arabian gulf, country of Bahrain, Am. J. Publ. Health 78 (1988) 314-315.

- [2] Arther R.G., Fitzgerald P.R., Fox J.C., Parasite ova in anaerobically digested sludge, *J. Water Pollut. Control Fed.* 53 (1981) 1333-1338.
- [3] Barbier D., Duhamel C., Perrine D., Georges P., Évaluation du risque parasitaire lié à l'utilisation agricole des boues résiduaires, *Bull. Soc. Fr. Parasitol.* 7 (1989) 259-264.
- [4] Barbier D., Perrine D., Duhamel C., Doublet R., Georges P., Parasitic hazard with sewage sludge applied to land, *Appl. Environ. Microbiol.* 56 (1990) 1420-1422.
- [5] Bergstrom K., Forekomst av parasittegg i avvatn 1981 fra koakkreinseverk og septiktankar in Noreg, *Nor Veterinaertidsskr.* 93 (1981) 323-330.
- [6] Bern C., Garcia H.H., Evans C., Gonzalez A.E., Verastegui M., Tsang V.C.W., Gilman R.H., Magnitude of the disease burden from neurocysticercosis in a developing country, *Clin. Infect. Dis.* 29 (1999) 1203-1209.
- [7] Blazek K., Schramlova J., Tissue reaction in experimental cysticercosis of sheep and goats caused by infection with *Taenia saginata* eggs, *Folia Parasitol.* 30 (1983) 131-139.
- [8] Boev S.N., On cysticerci of muscles of roe-deer in Kazakhstan, *Helminthologia (Bratislav)* 2 (1960) 47-54.
- [9] Borraz O., L'utilisation des boues d'épuration en agriculture : les ressorts d'une controverse, *Courrier de l'environnement de l'INRA* 41 (2000) 25-32.
- [10] Bouchet F., Léger N., Pollution parasitaire dans les boues des stations d'épuration des eaux usées de la région champenoise, *Bull. Soc. Fr. Parasitol.* 4 (1986) 13-18.
- [11] Bouhoum K., Schwartzbrod J., Quantification of helminth eggs in Waste water, *Zbl. Hyg. Umweltmedizin* 188 (1989) 322-330.
- [12] Bouhoum K., Habbari K., Schwarzbrod J., Epidemiological study of helminth infections in Marrakech waste water spreading zone. Second international Symposium on waste water reclamation and reuse, Iraklio, Greece, 14-20 October, 1995, pp. 221-231.
- [13] Brandt J.R.A., Geerts S., De Deken R., Kumar V., Ceulemans F., Brijs L., Falla N., A monoclonal antibody-based ELISA for the detection of circulating excretory secretory antigens in *Taenia saginata* cysticercosis, *Int. J. Parasitol.* 22 (1992) 471-477.
- [14] Cabaret J., Barbier D., Les risques parasitaires liés à l'utilisation des boues résiduaires urbaines : utopie ou réalité ? *Bull. Soc. Fr. Parasitol.* 19 (2001) URL <http://www.tours.inra.fr/sfpar/bulletin/bulletin.htm>.
- [15] Colloimb J., Baradel J.M., Thevenot M.T., Schwartzbrod J., Recovery of helminth eggs in sludge from wastewater treatment plant, in: Proceedings 3rd International Symposium on Processing and Use of Sewage Sludge Brighton UK, Sept. 27-30, 1983, pp. 230-231.
- [16] Corwin R.M., Bovine cysticercosis in feed-lots-Kansas, *Veterinary Public Health Notes*, Center for Disease Control, September, 1980.
- [17] Dada B.J.O., Taeniasis, cysticercosis and echinococcosis/hydatidosis in Nigeria: III - prevalence of bovine and porcine cysticercosis, and hydatid cyst infection based on joint examination of slaughtered food animals, *J. Helminthol.* 54 (1980) 293-297.
- [18] Deka D.K., Borkakoty M.R., Lahkar B.C., Cysticercosis in domestic animals in north eastern region of India, *Indian J. Parasitol.* 9 (1985) 83-85.
- [19] Devaux I., Intérêts et limites de la mise en place d'un suivi sanitaire dans le cadre de la réutilisation agricole des eaux usées traitées de l'agglomération clermontoise, Thèse de Doctorat, Joseph Fourier University, Grenoble 1, 1999, 333 p.
- [20] Doby J.M., Guillou L., Robin L., Gielfrich G., Fréquence de la cysticercose bovine chez le bétail dans l'Ouest de la France, *Méd. Mal. Infect.* 8 (1978) 334-338.
- [21] Dorny P., Vercammen F., Brandt J., Vansteenkiste W., Berkvens D., Geerts S., Sero-epidemiological survey of *Taenia saginata* cysticercosis in Belgian slaughter cattle, *Vet. Parasitol.* 88 (2000) 43-49.
- [22] Dudkowski A., L'épandage agricole des boues de station d'épuration des eaux-usées urbaines, *Le Courrier de l'Environnement de l'INRA*, 41 (2001) 134-135.
- [23] Dumontet S., Dinel H., Baloda B., Pathogen reduction in biosolids by composting and other biological treatments: A literature review, Proceedings of the 10th International Congress on environmental and sanitary problems in the Mediterranean areas, Vol. 1, Maratea, Italy, 10-13 October, 1997, pp. 251-295.
- [24] Duvaud E., Mugnier E., Gazzo A., Aubain P., Wiart J., Situation du recyclage agricole des boues d'épuration urbaines en Europe et dans divers pays du monde, Ademe Editions, Paris, 1999, 159 p.
- [25] Eckert J., Birbaum C., Abtötung von Wurmeiern in Hygienisierungsanlagen, *Schweiz. Arch. Tierheilk.* 125 (1983) 671-672.
- [26] Emery I., Bories C., Liance M., Houin R., In vitro quantitative assessment of *Echinococcus multilocularis* metacestode viability after in vivo and in vitro maintenance, *Int. J. Parasitol.* 25 (1995) 275-278.
- [27] Engelbrecht H., Mentzel U., Der urbane Wirtswechselzyklus von *Taenia saginata* im Kreis Wittstock, Bezirk Potsdam, *Angew. Parasitol.* 25 (1984) 126-132.

- [28] Fan P.C., Chung W.C., Soh C.T., Kosman M.L., Eating habits of East Asian people and transmission of taeniasis, *Acta Trop.* 50 (1992) 305-315.
- [29] Faust E.C., D'Antoni J.S., Odon V., Miller M.J., Peres C., Sawitz W., Thomen L.F., Tobie J., Walker J.H., A critical study of clinical laboratory techniques for the diagnosis of protozoan cysts and helminth eggs in faeces, *Am. J. Trop. Hyg.* 18 (1938) 169-183.
- [30] Feachem R.G., Bradley D.J., Garelick H., Mara D.D., Sanitation and disease: Health aspects of excreta and waste water management, New-York, John Wiley & Sons, 1983.
- [31] Fertig D., Dorn R., *Taenia saginata* cysticercosis in an Ohio cattle feeding operation, *J. Am. Vet. Med. Assoc.* 186 (1985) 1281-1285.
- [32] Forbes G.I., Collier P., Reilly W.J., Sharp J.C.M., Outbreaks of cysticercosis and salmonellosis in cattle following the application of sewage sludge to grazing land in Scotland, in: Proceedings of the 1st World Congress on foodborne infections and intoxications, 29 June-3 July 1980, West Berlin, Germany, 1980, pp. 439-440.
- [33] French Government, Arrêté du 8 Janvier 1998 fixant les prescriptions techniques applicables aux épandages de boues sur les sols agricoles pris en application du décret no 97-1133 du 8 décembre 1997 relatif à l'épandage des boues issues du traitement des eaux usées, *Journal Officiel* 31 Janvier 1992, pp. 6605-6612.
- [34] Fries G.F., Ingestion of sludge applied organic chemicals by animals, *Sci. Total Environ.* 185 (1996) 93-108.
- [35] Furet G., Birraux D., Wiart J., Deportes I., Epandage de boues d'épuration sur prairies et culture fourragères. Aspects microbiologiques, Ademe Editions, Paris, 1999, 155 p.
- [36] Gasnier N., Cabaret J., Moulia C., Allozyme variation between laboratory reared and wild populations of *Teladorsagia circumcincta* (Nematoda), *Int. J. Parasitol.* 22 (1992) 581-587.
- [37] Gaspard P., Schwarzbrod J., Helminth eggs in wastewater: quantification technique, *Water Sci. Technol.* 31 (1995) 443-446.
- [38] Gaspard P., Wiart J., Schwartzbrod J., A method for assessing the viability of nematode eggs in sludge, *Environ. Technol.* 17 (1996) 415-420.
- [39] Gaspard P., Wiart J., Schwartzbrod J., Parasitological contamination of urban sludge used for agricultural purposes, *Waste Manag. Res.* 15 (1997) 429-436.
- [40] Gauthier D., Rosaz C., Le Pimpec P., Lehy J.B., Valorisation des boues d'épuration de montagne - Risque sanitaire lié à l'épandage des boues en revégétalisation des pistes de ski. Évaluation et recommandations, Rapport de Synthèse, Conseil Général de Savoie, 1991, 37 p.
- [41] Geerts S., *Taenia saginata*: een eeuwig probleem?, *Verhand. Kon. Acad. Geneesk. Belg.* 52 (1990) 537-564.
- [42] Geerts S., The taeniasis-cysticercosis complex in Africa, *Meded. Zitt. Kon. Acad. Overz. Wet.* 38 (1993) 245-264.
- [43] Geerts S., Kumar V., Van den Abbeele, *Taenia saginata* cysticercosis in slaughter cattle in Belgium, *VI. Diergeneesk. Tijdschr.* 49 (1980) 365-374.
- [44] Geerts S., Kumar V., Mortelmans J., Sheep as an experimental model of *Taenia saginata* cysticercosis, *Trop. Anim. Health Prod.* 13 (1981) 37-40.
- [45] Geerts S., Brandt J., Kumar V., De Deken R., De immunodiagnose van *Taenia saginata* cysticercose, *Verhand. Kon. Acad. Geneesk. Belg.* 54 (1992) 329-346.
- [46] Geerts S., De Borchgrave J., Brandt J., Kumar V., De Deken R., Infectivity of irradiated and non-irradiated cysticerci of *Taenia saginata*, *Trans. R. Soc. Trop. Med. Hyg.* 86 (1992) 421-422.
- [47] Gonzalez L.M., Montero E., Harrison L.J.S., Parkhouse R.M.E., Garate T., Differential diagnosis of *Taenia saginata* and *Taenia solium* infection by PCR, *J. Clin. Microbiol.* 38 (2000) 737-744.
- [48] Habtemariam S., Catechols and quercetin reduce MTT through iron ions: a possible artefact in cell viability assays, *Phytother. Res.* 9 (1995) 603-605.
- [49] Hammerberg B., MacInnis G.A., Hyler T., *Taenia saginata* cysticerci in grazing steers in Virginia, *J. Am. Vet. Med. Assoc.* 173 (1978) 1462-1464.
- [50] Harrison L., Joshua G., Wright S., Parkhouse R., Specific detection of circulating surface secreted glycoproteins of viable cysticerci in *Taenia saginata* cysticercosis, *Parasite Immunol.* 11 (1989) 351-380.
- [51] Harrison L.J.S., Holt K., Sewell M.M.H., Serum antibody levels to *Taenia saginata* in cattle grazed on Scottish pastures, *Res. Vet. Sci.* 40 (1986) 344-346.
- [52] Harrison Z., McBride M.B., Bouldin D.R., The case for caution. Recommendations for land application of sewage sludges and an appraisal of the US EPA's part 503 sludge rules, Cornell Waste Management Institute, 1999, URL <http://www.cfe.cornell.edu/wmi/PDFS/landApp.pdf>.
- [53] Haugaard P., Cysticercosis and salmonellosis in cattle caused by sewage sludge on Danish farmland, *Waste Manag. Res.* 2 (1984) 163-168.
- [54] Horak P., Helminth eggs in the sludge from three sewage plants in Czechoslovakia, *Folia Parasitol.* 39 (1992) 153-157.
- [55] Hughes D.L., Norrington I.J., Morris D.L., Waite W.M., The effect of pasteurisation and stabilisation of sludge on *Taenia saginata* eggs, in: Inactivation of microorganisms in sewage sludge by

- stabilisation processes, Strauch, havelaar & l'Hermite eds., Elsevier, 1985, pp. 126-134.
- [56] Ilsoe B., Kyvsgaard N.C., Nansen P., Henriksen S.A., A study on survival of *Taenia saginata* eggs on soil in Denmark, *Acta Vet. Scand.* 31 (1990) 153-158.
 - [57] Ilsoe B., Kyvsgaard N.C., Nansen P., Henriksen S.A., Bovine cysticercosis in Denmark. A study of possible causes of infection in farms with heavily infected animals, *Acta Vet. Scand.* 31 (1990) 159-168.
 - [58] Kanazawa T., Asahi H., Mochida K., Simple techniques for preparation of small vesicles from *Echinococcus multilocularis* metacystodes and colorimetric quantitation of the viability of germinal cells, *Jpn. J. Parasitol.* 44 (1995) 441-446.
 - [59] Kerckhoven van I., Vansteenkiste W., Claes M., Geerts S., Brandt J., Improved detection of circulating antigen in cattle infected with *Taenia saginata* metacystodes, *Vet. Parasitol.* 76 (1998) 269-274.
 - [60] Khallaayoune K., Réutilisation des eaux usées en agriculture. Risque de transmission de parasites à l'homme et l'animal, *Hommes, Terre et Eaux* 103 (1996) 14-19.
 - [61] Khizhnyak N.I., Romanenko N.A., Disinfection of sewage sludge from helminth ova at the sewage plants of the town of Kiev. Parasitological problems, Proceedings of the VIII scientific conferences in parasitology of Ukraine socialist republic, Part 2, "Science Academy", Kiev, Ukraine, 1975, pp. 252-253.
 - [62] Kirichek V.S., Belousov M.N., Nikitin A.S., New data on the epidemiology of taeniasis in the extreme north of the USSR (data of investigations made in the Yamalo-Nenets autonomous region), *Med. Parazitol. Parazit. Bolezni* 6 (1984) 27-33.
 - [63] Klebanovskii V.A., Klimshin A.A., Shpil'ko V.N., The spread of taeniasis on the Yamal peninsula. Voprosy kraevoi infektsionnoi patologii. (Kishechnye infektsii i invazii.) (Tezisy dokladov.) Tyumenskoe Otdelenie Vserossiiskogo Nauchnogo Meditsinskogo Obshchestva Epidemiologov, Mikrobiologov i Infektsionistov, Tyumen', USSR, 1974, pp. 172-174.
 - [64] Kozakiewicz B., *Taenia saginata* cysticercosis in experimentally infected sheep and goats, *Med. Weter.* 33 (1977) 461-463.
 - [65] Kyvsgaard N.C., Ilsoe B., Willeberg P., Nansen P., Henriksen S.A., A case-control study of risk factors in light *Taenia saginata* cysticercosis in Danish cattle, *Acta Vet. Scand.* 32 (1991) 243-252.
 - [66] Lightowlers M.W., Eradication of *Taenia solium* cysticercosis: a role for vaccination of pigs, *Int. J. Parasitol.* 26 (1999) 811-817.
 - [67] Madeline M., Ballandonne C., Guyon R., Malas J.P., Vilagines P., Cabaret J., Barbier D., Mise en évidence de la viabilité des œufs de Taeniidés dans les boues résiduaires urbaines, *J. Eur. Hydrol.* 31 (2001) 85-90.
 - [68] Madeline M., Lendormy C., Guyon R., Malas J.P., Barbier D., Boues résiduaires – Réglementation actuelle et œufs de taeniidés, *Bull. Soc. Fr. Parasitol.* 18 (2000) 1. URL <http://tours.inra.fr/sfpar/bulletin.htm>
 - [69] Madeline M., Ballandonne C., Eldin de Pecoulas P., Guyon R., Malas J.P., Tourte-Schaeffer C., Cabaret J., Barbier D., Étude parasitologique des boues résiduaires urbaines: mise en évidence de la viabilité des œufs de Taeniidés, *Bull. Soc. Fr. Parasitol.* 19 (2001) 1, <http://www.tours.inra.fr/sfpar/bulletin/bulletin.htm>
 - [70] McCool C.J., Distribution of *Cysticercus bovis* in lightly infected young cattle, *Aust. Vet. J.* 55 (1979) 214-216.
 - [71] McGrath S.P., Chaudri A.M., Giller K.E., Long-term effects of metals in sewage sludge on soils microorganisms and plants, *J. Ind. Microbiol.* 14 (1995) 94-104.
 - [72] McManus D.P., Bowles J., Asian (Taiwan) *Taenia*: species or strain? *Parasitol. Today* 10 (1994) 273-275.
 - [73] Morris D.L., Hughes D.L., Hewitt R.J., Norrington I.J., Pathogens in sewage sludge: (ii) effects of sludge stabilization and treatment processes on viability and infectivity of beef tapeworm eggs, *Water Pollut. Control* 85 (1986) 476-481.
 - [74] Nansen P., Henriksen S.A., The epidemiology of bovine cysticercosis (*C. bovis*) in relation to sewage and sludge application on farmland, in: Block J.C., Havelaar A.H., L'Hermite P. (Eds.), *Epidemiological studies of risks associated with the Agricultural use of sewage sludge: knowledge and needs*, Elsevier Applied Science Publishers, London UK, 1986, pp. 76-82.
 - [75] Nelson G.S., Pester F.R.N., Rickman R., The significance of wild animals in the transmission of cestodes of medical importance in Kenya, *Trans. R. Soc. Trop. Med. Hyg.* 59 (1965) 507-524.
 - [76] Olsen J.E., Nansen P., Infectivity of eggs of *Taenia taeniaeformis* after anaerobic digestion of sewage sludge—a possible model for *Taenia saginata* egg resistance, *Acta Vet. Scand.* 31 (1990) 377-379.
 - [77] Pawlowsky Z., Schultz M.G., Taeniasis and cysticercosis (*Taenia saginata*), *Adv. Parasitol.* 10 (1972) 269-343.
 - [78] Pike E.B., Recent UK research on incidence, transmission and control of *Salmonella* and parasitic ova in sludge, in: Block J.C., Havelaar A.H., L'Hermite P. (Eds.), *Epidemiological studies of risks associated with agricultural use sewage sludge: knowledge and needs*, Elsevier Applied Science Publishers, London, UK, 1986, pp. 50-59.

- [79] Plachy P., Juris P., Transmission risk of helminth germs with sewage sludge to agricultural soil, *Slovensky Veterinarky Casopis* 19 (1994) 15-18.
- [80] Reilly W.J., Collier P.W., Forbes G.I., *Cysticercus bovis* surveillance-an interim report, Proceedings of the 8th International Symposium of the World Association of Veterinary Food Hygienists, Dublin, 30th August-4th September 1981, p. 298.
- [81] Rodríguez R.I., Differential diagnosis between *Taenia saginata* and *Taenia solium*; prevalence of *Cysticercus bovis* in the north of Ecuador, MSc-thesis No. 100, Institute of Tropical Medicine, Antwerpen, Belgium, 2001, 50 p.
- [82] Roy B., Tandon V., Metacestodiasis in north east India: a study on the prevalence of hydatidosis and cysticercosis in Mizoram, Nagaland and Assam, *Ind. J. Anim. Health* 28 (1989) 5-10.
- [83] Salm A., Erhebung zur Epidemiologie der zystizerkose des rindes im Raum Aachen, Doctorate in veterinary medicine Thesis, Hannover Veterinary Faculty, 1989, 93 p.
- [84] Schmidt G.D., Roberts L.S., Foundations of parasitology, Sixth edition, McGraw-Hill International Editions, New-York, 2000.
- [85] Schramlova J., Blazek K., Prokopic J., Some aspects of the presence of surface-associated acid mucosubstances on larval *Taenia saginata* infecting suitable and unsuitable hosts, *Folia Parasitol.* 37 (1990) 307-312.
- [86] SCVMPH 2000, Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on the Control of Taeniosis/cysticercosis in man and animals, http://europa.eu.int/comm/food/fs/sfp/mr/mr_sci_en.html
- [87] Semenas L., Brugni N., Viozzi G., Kreiter A., Monitoreo de parasitos en efluentes domiciliarios, *Rev. Saude Publica* 33 (1999) 379-384.
- [88] Sewell M.M.H., Haris L.J.S., Holt K., Serodiagnosis of bovine cysticercosis and the application of sewage sludge to pasture, in: Block J.C., Havelaar A.H., L'Hermite P. (Eds.), *Epidemiological studies of risks associated with the agricultural use of sewage sludge: knowledge and needs*, Elsevier Applied Science Publishers, London, UK, 1986, pp. 102-105.
- [89] Sinnecker H., The epidemiological significance of urban sewage sludge in the spread of possible zooparasitic infections, *Z. Ges. Hyg.* 4 (1958) 98-115.
- [90] Slifko T.R., Smith H.V., Rose J.B., Emerging parasite zoonoses associated with water and food, *Int. J. Parasitol.* 30 (2000) 1379-1393.
- [91] Slonka G.F., Matulich W., Morphet E., An outbreak of bovine cysticercosis in California, *Am. J. Trop. Med. Hyg.* 27 (1978) 101-105.
- [92] Smith C.T., Sylvicultural use of municipal and industrial biosolids: overview of USA research programs and research priorities. Biosolids utilization in forestry, Bordeaux, 29-30 March 2000, ADEME-INRA, Abstracts of communications, p. 4.
- [93] Stevenson P., Observations on the hatching and activation of fresh *Taenia saginata* eggs, *Ann. Trop. Med. Parasitol.* 77 (1983) 399-404.
- [94] Swiss Government, Ordonnance sur les substances dangereuses pour l'environnement, *Journal Officiel*, ordonnance sur les substances dangereuses Osubst., Modification du 16 septembre 1992, 16 p.
- [95] Thomaz-Soccol V., Paulino R., Castro E.A., Andreoli C.V., Helminth eggs viability in sewage and biosolid sludge in Curitiba, Parana, Brazil, *Arq. Biol. Tecnol.* 40 (1997) 829-836.
- [96] Thornton I., Abraham P., Soil ingestion as a pathway of metal intake in a grazing livestock, in: *Proc. Int. Conf. Heavy metals in the environment*, Edinburgh, Scotland, 1981, CEP consultants.
- [97] US Government, Part 503, Standards for the use or disposal of sewage sludge, 40CFR Ch1 (7-1-93 edition), Washington DC, USA, 1993.
- [98] Walther M., Koske J.K., *Taenia saginata* cysticercosis: a comparison of routine meat inspection and carcass dissection results in calves, *Vet. Rec.* 106 (1980) 401-402.
- [99] Wang I.C., Ma Y.X., Kuo C.H., Fan P.C., A comparative study on egg hatching methods and onchosphere viability determination for *Taenia solium* eggs, *Int. J. Parasitol.* 27 (1997) 1311-1314.
- [100] Watson D.C., Satchwell M., Jones C.E., A study of the prevalence of parasitic helminth eggs and cysts in sewage sludges disposed of to agricultural land, *Water Pollut. Control* 82 (1983) 285-289.
- [101] Wouters G., Geerts S., Brandt J., Kumar V., Calus A., *Meriones unguiculatus* as an experimental host for *Taenia saginata* metacestodes, *Int. J. Parasitol.* 18 (1988) 129-131.