Workshops on Echinococcus multilocularis on 1-2 April 2011

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1 April 2011: Workshop on *Echinococcus multilocularis* with special reference to the present Swedish situation

Time: 10.00-17.00
Place: National Veterinary Institute, Uppsala

1. Welcome and aim (Ann Lindberg/Maria Cedersmyg)

Background of the meeting today: The aim of today's work shop is to gain knowledge of possible ways to deal with EM in Sweden. By inviting experts from countries with a thorough knowledge of the parasite we hope to have a fruitful debate on possible ways forward. We are grateful to the Swedish board of agriculture for sponsoring this meeting. EM being a zoonosis is a shared responsibility between a number of authorities being risk assessors and risk managers. The reports on human health are based on; risk analysis, cost benefit and experts opinion

What action has to be taken regarding legislation and control?

2. AE in humans, diagnosis, treatment, prognosis. What are the expected risks for humans in the future in Sweden and in other European countries (Peter Deplazes)

EM is today regarded as an emerging disease; humans are not involved in the transmission biology of EM but can be regarded as accidentally infected. Lack of epidemiological data give rise to many tales to be circulating and journalists are interested. The susceptibility of humans is discussed , and is probably low, estimations showed that 1 out of 100 egg contacts -> disease.

Ways of transmission: Orally, mucosa, early development of oncosphere, the parasite is invading the liver, cavities histology alveolus (alveoli echinococcus).

The European Echinococcosis Registry (AE) 1982-2000: In total 559 cases of which 78% had symptoms
2010: In total 1060 cases (also more awareness)
Low incidence: 0.001-0.024

France has a low incidence, rare disease even in high endemic areas.
Studies in Germany showed that the reporting system is not perfect, underestimated incidence (3 times more cases?). Germany 50 cases every year probably more, 100?
Case finding studies in Switzerland 1956-2008, 94% found through hospital records, 20% found through serology (labs). Good reporting system with probably true figures.

Switzerland had an increase of cases last 20 years, especially the last few years but now it seems to have reached a new stable plateau. Higher incidence in females.

Detection in urban rodents focal distribution ex Zurich A. Terrestris 9%

EM protoscolices in 26 (3%) infected arvicola terrestris in Zurich.

Course of disease in the liver:

1) Dying of and calcification, extent unknown
2) Progressive growth and development of clinical symptoms; depending on size and location, median growth 14,8 ml/year. 5-15 years asymptomatic interval.

Symptoms: Unclear and unspecific. RUQP, Jaundice, painful jaundice….

Involved organs: 95% liver, metastases to other organs increasing with the age of infection (intraabd., lung, spleen, bone)

Diagnostic criteria:
Read: *Brunetti E et al, Acta Tropica 2010* about diagnostic criteria.
Imaging of lungs and CNS necessary. Typical lesions imaging, serology, histopathology
Imaging: High SE very good for prognosis and before surgery
Serology: immunofluorescence high SE lower SP, confirmation with specific tests

Treatment: *Torgesson et al 2008, J Hepatos*
1. Treatment of choice is radical resection of the liver followed by 2 years of high dose antihelmintic treatment. Surgery, how much of the organ needs to be removed? Not clear and a major problem.
2. Conservative with life time of antihelmintic treatment, high doses -> side effects.

In Switzerland treatment of disease is a huge success. Life expectancy has increased dramatically, now only 3 years lost for infected patients compared to the normal population. Average 54 years old when infected, do not know why. Disease development might be related to tumors. Children with infection are often immunodepressed. I.e. immune system has an important role.

Average age of infected persons is 54 years – why? No clear answer, maybe an immunological component or prone to develop cancer? Children that develop disease have been immuno-compromised.

In Switzerland treatment costs 100 000 euro per case (based on 155 cases)
Loss of income 78 000 euro
Total cost in Switzerland 2.0 million euro

Risk factors for infection (not many studies, *Kern 2004 EID*)

- Contact with definitive hosts, dogs, cats. Is dog the risk or is the risk assoc with habits assoc with the dog?
- Agriculture and forestry (63% of cases)
- Is dog the risk or is the risk assoc with habits assoc with the dog?
- Food -> vegetables, chewing grass, leaf or root vegetables, unwashed strawberries.No scientific data that says that population should not eat berries, same risk probably as buying salad.

➔ Hard to evaluate since incubation period is 5-15 years!

Dogs can be infected and has the potential to transmit EM, mean prevalence 0.3%. 9% of the dogs get infected at least once in 10 years. Today 1000 dogs infected in Switzerland, 10 000 in Germany. Cats unknown role, can excrete EM eggs but – number of eggs very low, not infectious?

EM Zurich: 10-15 foxes/km^2, EM detected in 19-60% of the foxes and fox feces everywhere in the city -> contaminates the whole city with eggs, strong infection pressure - but still there are very few human cases.

3. Ecology of EM in Europe (Peter Deplazes)

Major hosts; A terrestris, European Water Vole (easiest to catch and work with), M glareolus, Bank vole. Focal prevalence in Voles

Focal prevalence in voles. In Geneva and Zurich, A terrestris most common. EM protoscolices in 26 (3%) in Zurich. But can be a huge variation of worm burden in foxes.

Fox small tape worm:

- prepatency 26-37 days
- patency 2-3 months up to one year
- Very low rate of proglottisation (1-2!),
- mean number of eggs per proglottis: 200-300

EM reproduction in definitive hosts: foxes highest, high in raccoon dogs, lower in dogs and even lower in cats. One month later dogs still infected but foxes had a low worm burden. Different survival of the worms. Total egg production is comparable in fox, dogs and raccoon dogs, but low in cats.

Dynamic of worm population in foxes and dogs, depends on when the animal was infected. Only 10% of the foxes are heavily infected. What infections do you want to find? Predicted prevalence - be aware that you need to investigate more foxes.

Definite host fox + intermediate host rodents = Combination of survey in these gives higher probability to find EM.

Infection in foxes: higher in young foxes than in old (15%vs 85%). Recent studies in Baltic high in old foxes too, due to more rodents??

Netherlands – model to predict cases based on Swiss data: Annual incidence 15-20 cases in 2030

Lithuania – 2001 first detected EM in foxes, 2003 EM in 5 muscrats, 2001-2006: 58% prevalence in foxes. Maybe overlooked low prevalence before?

In humans, 128 new cases 1997-2010, incidence 0.2/0.5/0.8 /100 000 inhabitants, one of the highest incidences in Europe. Average age 57,7 years.

Rumania – prevalence in foxes of 3-15%, two putative cases. Theory: infection Austria-> Hungary-> Rumania. *Siko et al 2011, Parasite Res.*

Europe today: unknown in many places, endemic in many areas.

Future: More genetic analyzing of EM isolate with microsatellite. Data supp: Svalbard infected by 1-2 foxes from Central Europe.

Investigations: the border of endemic area, more interesting than central area. Distribution is different in different areas.

Not all rodent spices are needed but probably one of the major (needs confirmation). In some places M arvalis alone can support the cycle.

AE is a rare but emerging disease in Switzerland 10-28 cases/year. Huge increase of foxes in is probably the explanation to the increased incidence. New dynamic? Incidence increased initially in the rural areas, then in the cities. Convincing data show that the highest incidence increase is in now the high population areas (not cities).

Switzerland today AE in:
- Dogs more common than liver tumors in dogs, 10-15 cases/year.
- Primates very susceptile and die in AE infections
- Pigs occasionally lesion in liver different from milk spots, sharp surr. calcified, indication of AE

Need for pro-active public information programs:
- Priority to countries or regions where EM is prevalent, but rather unknown
- Achieve a realistic perception (avoid panic and prevent carelessness)
- Include young people and people living in urban areas to the target groups
- Include information about urban foxes and how to behave towards them (no feeding, keep them shy)
- Recommendations of options that are clearly related to the infection risk (ex. de-worming digs regularly)

4. Swedish situation (Helene Wahlström)

EM in Sweden:

- Screening until 2009 documented that Sweden was free from EM although a high probability of introduction (20%). Designed to find a prevalence of 1 %, also tested 0,5 % prevalence - 90% probability of freedom.
- 1st case female fox shot in December 2010 (egg isolation, PCR), 2nd case march 2011, adult female. These two cases came from the same place.

How was it introduced?
Probably by travelling dogs. Risk assessment showed that if not de-wormed travelling dogs -> a 98% probability of at least 1 infected dog per year. What is the probability of establishment? We do not know.

Sample size in Southern Sweden during 2011, sufficient to detect EM-with 95% confidence at a design prevalence of 1-40%. Assuming a test with 90% sensitivity

- 4 counties- 10 foxes per municipality
- The rest: 4 foxes per municipality

- 120 feacel samples dogs (mainly hunting dogs); 110 analysed and negative
- 3000 submitted foxes 1000 have been analysed, one positive (second case)
- Traps rodents initiated
- 4 municipalities -> rec de-worming of dogs

Routine was 300 foxes per year before the first case in December 2010

Diagnostic methods:
Until 2009: Copro antigen ELISA
After 2009: Egg isolation and PCR
After positive finding: SSCT

Future surveillance?

Design prevalence:
Given the samples we have taken, we can find a prevalence of 1% to 40% depending on area. Would like to sample more (as planned per municipality), designed to find a prevalence of 1 to 10% depending on area.

Focus on: Sampling the infected area? Identifying borders of infected area? Sampling to identify free areas? How much effort should we take, to what costs?

Wild life:
Fox population density 0,2-0,4 adult per sq km infected place
0,8 adult per sq km in the south
Do we have urban foxes? Probably but not high pop dens.

Rodents:
- Population of Wild boars are increasing, esp. around coast but not in the infected areas
- Bank vole, Water vole, Field vole are common
- Common vole – not in Sweden
- Muskrat, in the very north of Sweden

A. DISCUSSION

Q: What is 5-15 years incubation period based on?
A: 2-3 papers. Observations of patients that have moved from endemic area together with knowledge of growth rate lead to the estimation of 5-15 years. Anecdotes and not really proven. Incidence very low, hard to do a study on this!

Q: Lithuania how where the people infected?
A: Ongoing study collecting basal data, rural population, many retired.

Q: Underreporting, 3 times higher estimated that the reported cases. The unreported cases- did they not have any symptoms?
A: Other investigations discovered occasional lesions from EM. Some cases became clinical after that, psychology?

Q: Seroconversion in humans?
A: 5% of the cases seronegative. Antibodies – specific can often be detected, no relation to other findings. Follow up of seropositive persons – many do not develop any symptoms. Parasite dying out? Longer incubation period? Recommend to follow up these persons for a long time. Repeated infections necessary? Perhaps combination genetics and infection pressure.

Q: Metastasis?
A: Small cyst probably with laminated layer around. Even single cells can infect new animals, multipotent cells.

Q: Finland? Why not there?
A: Foxes became almost eradicated in Finland - lack of host?

Q: Eradication by baiting?
A: Depending on: Precise coordinates of foxes infected? Single focus or several? Size of focus?

Denmark: Local prevalence very high. No surveillance on dogs - would have been preferable, like Sweden is doing now. The discussion where it came from did not start, probably from the Baltic regions? No border control. Boat traffic? DK stopped reporting human cases in 2006.

B. DISCUSSION

Q: Switzerland: Awareness of the risk of infection?
A: No, not really. No longer monthly information of EM, as before.

Q: Program investigating exposures in different countries?
A: There are some programs, China, Kazakhstan and Kirgizstan since high prevalence but very different incidence in human cases. Different behavior regarding dogs and different food culture. Difficult to study this in Europe – lack of good incidence data.

Q: How the parasite came to Sweden; Perhaps rodents? Imported hey?
A: Cannot be excluded but dogs are probably a higher risk (based on risk assessment).

Q: Role of dogs, uneven distribution although in all areas people has dogs and people travel. How important are the dogs?
A: Dog owners today not so concerned because no border control. Microsatellite analysis of isolate for tracing would be beneficial! Relationships dogs and foxes - Germany: Probably massive underreporting but not a big role of dogs. Prevalence in foxes 20-25% in one investigated area and no dogs in the same area where infected, many dogs tested.

Q: Germany 20-30% prevalence in foxes but no human cases, other areas same prevalence and human cases. Why?
A: Dogs clearly a risk factor but might be different in different regions.

Q: Risk mapping globally collecting data from different populations, density, rodents, foxes -> to map out hot spots? Is that possible?
A: Needs an enormous amount of data. Many ecological data are unclear a very local level necessary.

Q: Mean age infected people (54), different in urban areas?
A: In Switzerland no differences between areas or sex. In China there is a lower median age and also a tendency in Kirgizstan but not in Lithuania.

5. Tests for EM, Se, Sp and possibilities of pooling? (Peter Deplazes)

Expensive! How decrease the costs?
CoA: lower Se than dissection of animals. A less sensitive method needs an increased number of tested animals.

Low - high infected animals. If rodents are infected there are high infected foxes present even in low infected areas.

Time aspect:
- 100 samples/day CoA
- 15/day egg isolation
- 20 foxes/day necropsy team (depends…)
- Smear test perhaps 40/day.

Money and Se!

SCT – best Se, > 95%
Smear test Se 75% (70-90% dep on quality of material)

Egg isolation - spend a lot of time for egg isolation not too much on DNA isolation
Direct DNA isolation; lot of work, inhibition reaction, probably go back to the egg isolation.

CoA
- Se dependent of worm burden > 50 worms, good Se 60-70% < 50 worms ap. Se 50%
- Sp 100%

If killing too many foxes, new foxes will move in, is contra productive to a stable fox pop.
System for collecting fecal samples instead, antigen can keep up to 2-3 weeks, but fresh is better, older feces risk of bacterial growth. Eggs can be isolated even from very old feces.

C. DISCUSSION

Q: Health hazard for laboratory staff?
A: All material treated in -80 for 3 days before handling. If a whole fox 4-5 days.
Routine analyses for dogs –not freezing because that will destroy other types of parasites as protozoa etc.

Q: In what way can Swedish foxes differ from other foxes?
A: Regarding Se of the test. Lower egg burden? Lower worm burden? Function of intermediate host – could be lower infected. Unknown!

Q: Seroconversion in humans?
A: No studies for humans. Exp inf. in pigs antibodies detectable after 4 weeks, mice 6 weeks. It is probably similar for humans, early antibody reaction? The dynamics is unknown.

Q: Se on egg PCR? If Pooled? (Estimated 50% one in study. Norway uses pools of three. Sweden now uses Se 50 % for a pool of 3, is that OK?)

A: Egg number can be very low, 5 g should be used not 1g, level of detection. Don't like the idea of pooled samples. Many cases only single eggs detected so a level that is directly related to the amount of feces.
Low Se linked to flotation and prepatent period (no eggs)
One egg is enough for positive PCR (can have many worms but no eggs)

6. Presentation Time-space analysis of EM-infection in foxes (FJ Conraths)

Human cases in Germany, EG 67, EM 26 (EFSA Journal 2010). In 2009 25 cases, 2010 30 cases. Probably 2/3 underreporting, so probably 60-100 new infections per year. Some may be due to already infected Russian immigrates. EFSA/ECDC figures show high incidence for Germany, only exceeded by the Czech-republic.

In 2007 6523 foxes were analysed for EM of which 22,9% were positive. There are large differences between regions (federal states). High prevalences in the south, medium in the south-west and east, low in north.

Country (in the case of Sweden and Germany) is a too large geographical unit in order to get reliable data on number of foxes to be analysed in order to identify presence of infection. You have to design surveillance in smaller geographical units. Problem also when endemic foci are small. Detection of small foci requires large sample sizes. Major challenge if many small foci in a country. Other problems: Spatial heterogenicity, Random variation (small numbers of samples per spatial or temporal unit, missing data in spatial or temporal units) and negative effect on precision of prevalence estimates.

Approach to be used: Hierarchical Bayesian Spatio-temporal model (presentation of use of model in Thuringia and Brandenburg). Model works in some communities, not in others. Depends on the spatial distribution?

Monitoring: Sample size should be calculated with reference to local red fox and raccoon dog population. At least 300 foxes / spatial unit raster should be sampled (for spatial units of 25,000 square km) (95% - 1%).

Detection of small foci is a problem. Potential solutions (Working document SANCO):

-Detection over time. Disadvantage: zoonotic potential may remain undetected for a long period of time.

-With increased monitoring effort. Disadvantage: high costs

D. DISCUSSION

What geographical area should be sampled in Sweden? Consider regions where a certain human population density (maybe cynical…) and fox population density is present.

Do we know the cause of variation in prevalence? No, it is only known that the endemic area has increased in Germany. This might be due to the fact that the fox density has increased after eradication of rabies. Infected foxes are more common in areas near water (maybe because intermediate hosts are there). They are also more common in agricultural areas than in forests, maybe because voles are more abundant in the first-mentioned. Are urban foxes a problem? Not in Germany, no positive foxes have been found in Berlin, maybe because they feed on garbage and do not need to eat intermediate hosts. In the surrounding (suburban) areas of the cities, where infection is found, foxes are both well fed and come in contact with intermediate hosts.

It is difficult to say which intermediate hosts are most susceptible and effective in spreading the infection.

What is the objective of the surveillance that is in place? Monitoring or intervening? Decided at which level – region or local?

Will Germany continue monitoring even if it is decided that nothing can be done? The government has recommended states with high prevalence's to reduce monitoring, there´s no point in putting effort into that any more.

Has there been any rodent studies or have studies only been focused on raccoons etc? One student has done some work on rodents but it is difficult due to their heterogeneous spatial distribution. It is probably not meaningless to focus on rodents, but it is difficult to come to any conclusions that are useful from an epidemiological point of view.

Could the infection in the rodent effect its behavior and make it more susceptible to predators? Difficult to answer.

Biodiversity studies show that the less the biodiversity the higher the prevalence of infection.

Are there any differences in reservoir competence between rodents? Are there threshold densities for foxes and intermediate hosts?

Are there any groups that have worked on models of infection dynamics? Yes, there are some (Hans Tulkhe), Franz shall look into that.

Is it possible to eradicate the parasite in Sweden? How do you know if a new finding is a new infection or due to unsuccessful eradication?

To eradicate you have to be sure that this is an isolated foci. If there are several foci you can forget about bating (or it will be very expensive…). In an area of 5,000 square km you could do bating every 6th week in the beginning and then every 3rd month and maybe be back to normal after 3 years. However, Franz personally believes that there is no point in trying to eradicate the parasite in Sweden, also in the light of the fact that the disease is uncommon in humans and that there are no human cases here. We should continue monitoring in foxes, of course there is a risk that it will be too late to do something if the prevalence in foxes turns out to be increasing.

Would the recommendation be the same if we could be 100% sure that we could prevent re-introduction? It is probably very difficult to be sure about preventing reintroduction, the present situation in Sweden shows that. De-worming is only effective for a short period, maybe not very useful to de-worm 10 days prior to entry. There is an ongoing discussion in Brussels about allowing de-worming less than 10 days prior to entry.

Are the Germans happy with the intestinal scraping technique? Yes, but the SCT is the standard.

What about meat inspection and wild boars, are there any lesions found at slaughter in Germany? No lesions reported. In Japan, meat inspection of domestic pigs is used in surveillance, couldn´t it be used here?

Can we target the sampling of fox feces? They defecate all over the territory. Feces easy to find at places where they predate/eat, at places with landmarks such as e.g. a tree in a field.

Contact with earth and vegetables seem to be risks, why should not berries be a risk? Maybe they are, there´s just no evidence. What should the recommendations be about kindergartens going out to pick berries in the woods? Information important, then everyone has to decide about what risks they are prepared to take. If you do not want to take any risks, you should not eat un-cooked berries. Serological screening of hunters in Switzerland did not show a higher prevalence in them than in the normal population. Young people have a lower incidence in general. What is the number of immunosuppressed people in Sweden? Immunosuppression does not increase the risk of getting infected but of developing disease. This should be considered as an opportunistic infection.

Important to highlight the fact that it is different for us to relate to the disease since we have a culture where we are used to be able to go out in nature and pick berries etc safely, and since it is new to us. It is important to emphasize that this disease is very uncommon in humans. It is probably very difficult to prevent re-entry of the disease even if we could eradicate it now. Yes, we have rules, but it is the compliance of the rules that matters.

If you want to try to eradicate the parasite, you should do it very quickly, otherwise it will be difficult. There is actually no evidence that it will re-enter for sure after eradication.

Contribution of wildlife to infection? Wolves? There are about 220 wolves in Sweden. They probably pose a low risk since they are not focused on rodents. It is probably most important to look at raccoon-dogs.

What about transmission of the disease by water, aerosol, dust? The eggs are susceptible to dry conditions and sun, spreading by aerosol unlikely. Freezing does not kill the eggs (-20 degrees). Eggs survive well in water.

7. Practical experience of baiting, could eradication by baiting be done in Sweden? Daniel Hegglin

Cannot answer the question if eradication by baiting can be done in Sweden.

Our experience is special, and differs from the situation here. We have urban foxes.

Incidence of human AE increases as the fox density increases in urban areas.

Energy expenditure per adult fox: 876 MJ/year. One household is enough to feed four foxes.

Fox home ranges: females 9.8 ha, males 13.4 ha.

High prevalence of EM in foxes in periurban areas.

Factors affecting the urban cycle of EM & the infection pressure with EM eggs. There are different factors within each of the following areas:

1. Forest
2. Agriculture & recreational
3. urban periphery
4. central urban area

What can we do to avoid infection

- De-worm dogs
- Hygiene, wash / cook food, wash hands, avoid contact with foxes.
- Prevent dogs from predation on rodents
- Fox population, culling, food, changing behavior
- Regulate intermediate hosts

Regulation of fox population:

- number of juveniles high & variable
- high mortality during the first year (compensatory mortality)
- animals without reproduction (flexible social structure)
- shooting influences spatial dynamic and age structure: increased dispersion, larger home ranges, movements between territories, higher amount of juveniles

How to control EM? Rabies vs EM

	Rabies	EM
Vaccination	+	-
Infection after treatment	-	+
Free living stages	-	++

Studies have been done on baiting EM. Decrease of prevalence.

Baiting study in Zurich: Baiting in city periphery. Different baiting strategies.

Second year: Decrease of prevalence in the intermediate host.

Baiting every 3 months not so effective.

3,5 years after baiting there's an effect in the area monthly baited.

Recommend baiting every 4 weeks.

If you are thinking of eradication, best to start immediately, before young foxes disperse.

Eradication scenario:

Baiting area: radius 4x mean HR diameter (e.g. 12 km ~ 450 km2), 30 baits/km2, 4 w interval, ~180'000 baits/y, 4y)

Control area: radius 10x mean HR diameter (e.g. 30 km ~ 3'000 km), no baiting, sampling without fox and rodent collection.

Monitoring without fox culling

- Fox feaces
- Targeted rodent trapping (muskrats as sentinels, arvicolids in baiting area)
- Wild boar

If you consider to do something like this, it's important to decide how to sample.

It could be dangerous to shot foxes in the control area, since foxes from the baiting area can disperse.

Foxes probably eat rodents even if the foxes are given supplementary feed.

Important to deliver the baiting in space.

Cost calculation: bait ~ 130 000 Euro per year.

Conclusions:

- Information should be considered as an important tool for prevention
- Monthly anthelmintic treatment of dogs with possible access to voles
- Control by regulation of intermediate and final host is not promising
- Fox culling must be implemented carefully -> effect on spatial behavior
- Anthelmintic baiting is expensive but a feasible control option and extinction is realistic in a very localised focus (intense and long lasting baiting necessary)
- Scenario should planned if other foci will be detected
- Ecological data required: fox densities , home range sizes, dispersion distances, predation rates on intermediate host species

E. DISCUSSION, what actions can/shall be taken in Sweden?

Is there anything like fox repellents? All means I know have short term effect, hard to do on large scale.

Is it always the case that foxes prefer the bait? Even in areas that have all lot of food, baiting is consumed.

Airplane dispersion, what is the precision? Have no information. Have been done in Germany.

Has this bug been eradicated anywhere? You will not be able to show this.

How far from eradication have any study been? It has been reduced from a high prevalence to below 1%.

One problem is that hunters might increase the hunting of foxes in the infected area and that this can affect the dispersion of foxes.

Major problem to determine the size of infected areas. If we shot foxes, the dispersion of foxes might increase.

In Denmark: It has been accepted that EM exists. The Danish authority was not willing to invest in eradication.

From a cost benefit view, should we focus on this disease or not. It's not easy to give an answer on costs and so far there have been zero costs on the human side.

From a Swedish - right of common access - perspective it can also be costly.

Is it better to invest the money now to try to eradicate it, then to take the costs of human cases in the future?

What is the probability of spread of EM, some foxes moves long distances.

De-worming of dogs must be an important part of a baiting, otherwise difficult to be credible with de-worming of dogs at the Swedish border.

Baiting in peripheral urban areas has shown to be cost effective.

Can we predict the EM infection 10-15 years from now? Important to develop models to help understanding the possible spread. How much information can a model give us? There's a lot of uncertainty in a model. Is there any threshold value when it is too late to do anything? Impossible to answer that question, but studies have shown a substantial decrease in high prevalence areas.

Isn't this a good candidate for wild life monitoring? We should be able to afford costs for baiting and monitoring.

Public perception: What will the journalists write if don't do anything? Depends much on how we present the case, there are no experience of eradication anywhere in the world. Will the tourism be negatively affected if we don't eradicate? Tourists from Germany call SVA and ask if EM exists in Sweden.

It's a very complex problem where there are many gaps of knowledge. It would be very valuable to compile all options, such as eradication, monitoring etc. To have more and better information for decision, it would be valuable to study perception given the options.

When is it too late to do anything? And how long can we wait? Differences of the situation in various countries

Money is not the main problem, if EM only exists in this area, but there are many unanswered questions. We must understand the risk for reintroduction of the infection. And if we don't do anything, how do we handle that.

A pragmatic try would be to do it as a research study with focal baiting and monitoring. Foxes migrate during September to January and some individuals move up to 100 km. It would be best to do something during early autumn.

How many cases can be expected in Sweden? With the same incidence as in Switzerland, about 26 cases. There will be substantial costs and suffering for these cases. Some costs cannot be measured.

2 April 2011: *Echinococcus multilocularis* workshop with focus on strategies and future research

Place: National Veterinary Institute, Uppsala

Present:

Helene Wahlström

Ulla Carlsson

Dan Christensson

Erik Ågren

Henrik Uhlhorn

Gert Olsson

Birger Hörnfeldt

Peter Deplazes

Daniel Hegglin

Christian Kapel

Secretary: Aleksija Neimane

HW opens workshop with her thoughts on topics she would like to address:

-baiting

-surveillance, test

-no baiting?

-biotope modeling- what will happen?

-age distribution AE

-future research (risk factors)

First presentation: Christian Kapel on the Danish experience

NB: Refer to the electronic copy of his presentation for details, references, figures.

Parasitological studies in Danish foxes
-no true surveillance in Denmark

-120,000 foxes

40 000 hunting

-30 000 road killed

-2-3 foxes/km2

-5-6 foxes /km2 in area around Copenhagen (CPH) (now 1-2/km2 because of mange outbreak?)

EÅ- asked about using road kill during non-hunting season for surveillance

CK: In Denmark, all work is voluntary and/or done by PhD students, so data was collected through hunters association, road kill, etc

-focused on area around Copenhagen, but had foxes from all of Denmark

-In 1994, there was serological indication that EM might be present 10% (of 145 foxes), whole country

-so went ahead and did some surveillance in foxes

-In 2002: necropsy of foxes: 3 (possibly 4) of 430 foxes from Copenhagen positive (1% prevalence), no foxes positive from other areas (but focus of sampling on CPH)

-Saeed et al (2006): examined 1040 foxes (436 roadkills, rest from hunters). Found EM in 0,7% in CPH foxes, not in the rest. (AN comment: 0.7% is equivalent to the 3/430 positive foxes from Copenhagen mentioned above. Seems like the necropsies were performed in 2002 and then results published in 2006?)

Methods: Sampled foxes from 1997-2002 from all of Denmark (focus on CPH- 10X more numbers assessed from CPH than other areas)

Basic age determination (3 classes)

-Post mortem and EM sedimentation

-did a survey for different parasites: of 430 foxes from CPH, found 3 (maybe 4) positive foxes (see above)

-all rest were negative for EM

-looked for various other parasites as well

-media became alarmed, EM results got attention, but no discussions with veterinary authorities (seemingly ignored the situation)

-however, got money from research council for some more work, so these initial results raised awareness

-in kinder gardens, for eg, now cover sandpits with nets over night

-historically, small numbers of foxes examined for parasites in other studies

-can only say that EM is present in Denmark, there may be changes

-discussions about cats- are they a risk for human infection?

Did a risk assessment (Kapel 2006)- infected raccoon dogs, cat, foxes, dogs (20 000 live larvae each i.e. per animal)- after 1 month, all species excreted eggs, except not many eggs excreted

from cat (no difference between egg excretion from raccoon dogs and foxes regarding amount excreted, but raccoon dogs excreted over a longer period of time). Dogs excreted smaller numbers than fox per time unit but continued to excrete steadily for much longer time at least 70 days,(*contrary to other information, we should check reference*)

-other species excreted more than 100 000 eggs, but cat just 117 –suspect cat a dead-end host

Raccoon dog: 201 485 per animal, fox 189 682, dogs 151 850 (average number of eggs in feces per animal)

-program in Denmark to exterminate raccoon dog (now raccoon dogs probably all over Jutland) –have been introduced and want to get rid of them (extermination program doesn't get much press because controversial re: extermination)

HW: as raccoon dog population expands, will it affect EM biomass contribution?

PD: we are looking at Lithuania because they have both raccoon dogs and foxes- prevalence in raccoon dog in eastern Europe always much lower than fox (eat more amphibians than rodents)

-raccoon dog more a downstream definitive host. If high prevalence in foxes, local raccoon dog also infected-believe raccoon dogs use latrines, so contamination of environment much more localized. Hypothesize that raccoon dogs not as good a definitive host/spreader/as significant re. EM as foxes

-raccoon dogs are excellent hosts for all dog and fox parasites (e.g. Alaria- raccoon dogs are exceptional hosts) (and Alaria has some water association, and raccoon dogs eat lots of amphibians. Need more data about feeding ecology of raccoon dogs)

HW: raccoon dog feeding habits differ in Finland than in rest of Europe. Lower prevalence of EM in raccoon dogs than foxes in general

PD: hibernating raccoon dogs- empty GI tracts, no parasites. Question: What happens to parasites over winter?

CK: Must remember that there is little evidence for a lot of these hypotheses e.g. the hypothesis that raccoon dogs are 'highly susceptible' to EM perhaps is not well proven

-discusses a rodent study done in Denmark:

(Al–Sabi in prep) 719 rodents trapped in Zealand (25km around CPH)- species not specified here

Rural forests (n=340)

Urban forests/parks (n = 211)

Residential gardens (N=168)

126 rodents had liver lesions. Looked at lesions using PCR and sequencing: 94 had Taenia (T. taeniaformis, T. mustelae and T. polyacanthae), but no EM found

-the 3 positive foxes found were all south of (or in southern) CPH in urban parks, within 10km of each other (not a forest area, but a park area- finding of EM was a surprise) –likely an introduction to local rodent population and foxes then got infected

-other observations: no positive foxes in roadkills examined from 2005-2010, (CK doesn't know sample size because no reports available. Work done by Veterinary Institute)

Dyachenko et al (2008)- 1 cat fecal sample reported positive, but results questionable (lab contamination?)

Studies begun in 2011: raccoon dogs: extinction program, southern Jutland (not around CPH). Will examine them for EM

Cats: Tritrichomonas fetus study in north Zealand, will look for other parasites at the same time

-nothing planned on foxes- seem largely ignored. Why? CK doesn't know

-CK also unsure of methods that will be used in above studies, but believes they will use egg isolation PCR/coproPCR on feces

EÅ: We look at all Finnish raccoon dogs that come over here to Sweden at SVA

CK: Brings up another example of parasites shared by dogs/foxes:

A. vasorum among dogs is emerging (Zealand, Denmark in CPH area)

-clear link between cases in dogs and cases in foxes (emergence)

See CK's presentation for references. No reports of A. vasorum in foxes or dogs as late as 1983. Now up to 92.8% prevalence in foxes around CPH (Northern Zealand)

In terms of EM, potential studies (data for risk assessment) needed in DK and are being planned:

-prevelance of EM in foxes?

-Incidence in dogs?

-Susceptibility of different rodents?

-Rodent ecology?

-makes the point that rodent communities differ in s Sweden compared to DK

HW: if dogs eat fox feces, may be coproPCR positive. This is a false positive regarding infection (But general consensus is maybe it doesn't matter, as it gives an indication that EM is present)

PD: if you feed a dog eggs, the eggs are directly infective (refers to old study by Jenkins). Can measure an immediate immune response (eggs may hatch, elicit immune response, then eggs die). Eggs may survive passage in dog GI tracts

-cat studies on feces are biased because easiest to collect fecal samples from indoor cats! (not collected from outdoor cats for the most part, so wrong cat group targeted)

-if you find a positive dog, would have to go back to dog, and test it again. If dog is still positive, dog may be truly infected (versus just ate eggs that are passing through)

EÅ: dogs as intermediate hosts- is it correct that more liver damage in dogs is caused by EM than even liver tumours? Potential worry for Swedish hunters regarding hunting dogs licking on dead hunted foxes and eating eggs, then becoming intermediate hosts

PD: Clinicians in Switzerland: if dogs present with destructive liver lesions, EM is high on differential list (dog as an intermediate host). They don't tell the public that the dog can occasionally be an intermediate host, because this is very confusing. Over the years, have seen 30-40 cases of dogs as intermediate hosts, and in some of those cases, dogs also had intestinal infection. Question: Did the dog get the infection from fox eggs or from its own infection? Also found EM material in dog bile in one case- possible route of infection from liver to intestine in dogs? Speculation.

-discussed available serology test: based on a reaction to the oncosphere. Have had foxes with lots of worms that are seronegative, and foxes that are highly seropositive with no worms. Test no longer done/used by PD (but still exists). Basically, serology is not a good test to detect intestinal infections.

HW to CK: what would you have done in DK if you could?

CK: do prevalence studies every 5 years (with good sample sizes). Because so low prevalence, risk to people is minimal. CK would like to see prevalence tests regularly in foxes throughout Denmark. Cost also not hugely significant. Would be interesting to repeat study now that Sweden has positive foxes. Prevalence every year not necessary. Denmark has accepted that there is a small risk, and if the prevalence increases over 5-10%, then maybe need to take action. To bait in the countryside now with this prevalence is not justified.

HW: if EM found only in CPH, would you want to get rid of it?

CK: If introduced once, could be introduced again, so extinction is tough. Better to know the risks

PD: Denmark had a focus, and it may have died out (why? Perhaps circumstances not ideal so the parasite was not perpetuated, may have died out).

CK: Denmark is Sweden 10 years ago. Denmark did nothing- what happened? Would be great to go back and see if EM died out on its own.

HW: but the biotope in Denmark is different from the biotope in the Swedish positive area. Also, the rodent community is very different. What are the risks of establishing the life cycle of EM in the Swedish biotope? Do we have the right rodent hosts? If not- EM will die out. May be introduced again.

CK: In Denmark- some indication that rodent communities are changing. Same in bird communities- related to climate change. Sweden would provide new and unique data to this hypothesis. In Denmark, in rodent communities, believe species composition is changing

GO: in Belgium, get rodent irruptions with masting of beech and are seeing similar things now in Sweden

DH: Important to get prevalence data. But two hypotheses 1: EM pops up, may spread 2. EM present and reoccurs every few years. Believes baiting is a reasonable approach. Can never prove that baiting eradicated the parasite, but if the second hypothesis is correct, can have a chance to get rid of EM. With such a low prevalence as in Sweden, if bait for 4 yrs and then have no parasite, don't know if baiting made a difference, but does it matter?

PD: Gives an example of a stable EM focus in Switzerland: Small valley in south. Endemic area for EM. Not moving southward because EM is linked to Microtus populations, and these rodents don't exist south. So EM is very stable here (rodent community limits spread of parasite)

HW: If the parasite disappears from an area, something must have changed in rodent population?

DH: or there may have been local environmental changes e.g. few dry hot years

BH: Can get high rodent numbers in southern Sweden. Last year was a very good rodent year in Sweden (southern Sweden)

GO: Two rodent dynamics in Sweden: one in north, one in south. No rodent cycling in south, but get irruptions because of increased feed availability e.g. beech/oak masting. No indications that this year will be a low rodent year in Sweden.

BH: Plus the stable snow over Sweden this past winter was good for rodents

DC: Do rodents eat fox feces? If so, which rodent species tend to do this? Why are only some rodent species infected with EM?

PD: If a fox is marking rodent habitat (even on burrows), there will be high egg density in soil. Rodents may ingest eggs that way. Intranasal exposure experimentally has caused lung EM instead of liver EM in rodents. However, rodents need to ingest eggs for liver infection (i.e. proper infection).

CK: Just rain on feces spread eggs to surrounding vegetation. Rodents don't have to actively eat feces

PD: In the Taenia transmitted by cats- up to 20% prevalence was found in rodents. Therefore rodents have to be ingesting proglottids, but don't know if rodents actively eat feces, or just contaminated food. Answer of how eggs get into rodents is unknown.

HW: Points out there is an old experimental study where lots of different animal species are infected with EM, but didn't have reference off the top of her head.

Second presentation: An outline slide of scenarios presented by DH

Eradication scenario (hypothesis: focal establishment)

-infected foxes

-baiting area: radius: 4x mean HR diameter (e.g. 12km ~450 KM2)

30 baits/km2 at 4wk interval, 180 000 baits/yr for 4 yr)

Sampling with fox and rodent collection

-control area: radius: 10X mean HR area (e.g. 30 km ~3000 km)

No baiting

Sampling without fox and rodent collection

-monitoring without fox culling

Fox feces

Targeted rodent trapping (muskrats as sentinels, arvicolids in baiting area)

wildboar

DH: need to monitor that foxes are eating baits (not wild boars/other animals). They use cameras.

-will need/get lots of data on fox home range, density, etc that is necessary

-baits aren't meat-based. Wild boars, hedgehogs find bait very tasty. Cats won't eat baits. Photographed stone martens and badgers around baits, but have never seen mustelids eating bait. Sometimes hard to see if bait just checked or actually eaten. Don't see birds (e.g. corvids) eating baits either. Use reflectors on baits so show up on photographs. Rodents and snails can eat baits if baits put out in summer and if bait sits in the area for a long time

HW: fox HR is 5 km2

DH: 4x HR diameter is just a suggested starting point re: baiting area

PD: Points out that the two positive Swedish foxes are dependent events. Asks if they were found at the same spot? Yes.

EÅ: Both foxes were found at same hunting place, but tested foxes from all over, not just from one same place.

PD: so good evidence that EM in Sweden is truly a localized event? Probably

EÅ: Have shot 12 foxes on the property, and many more in surroundings

GO: also have fox den location information in the area of positive foxes. Will give an indication of where to focus efforts for rodents /voles

DC: E6 highway runs between the coast and the positive fox site. DC has hard time figuring out how introduction occurred in the middle of a forested area. (But Uddevala, the port, is on the same side of road as the positive fox site)

HW: 10km from Uddevalla (port) to place of positive foxes.

DH: 12 km radius of baiting would reach Uddevala (big port).

DC: The E6 is also heavily touristed. Tourists to Norway, etc use the E6. E6 may be a barrier to foxes? (At least lots are run over here)

EÅ: Also get Danish hunting dogs coming in and entering the forest. Another way of introduction?

UC: Also German hunting tourists come into this area

PD: Suggest Sweden puts its effort into collecting fox feces (500) in the area of positives to try and establish EM range. Are two foxes the centre, or e.g. the northern limit of EM in Sweden? Thinks this is the most efficient way to try and map extent of EM focus in Sweden. Now not the best time of year to find fox feces. In Switzerland, it's easiest in autumn to find fox feces

GO: Thinks it might be easiest now to find fox feces because no vegetation and snow has melted away… In Sweden, can hunt foxes out of season if good reason. Can take fox pups locally once they emerge from the den.

HW: But too expensive. More cost-effective to collect feces. Don't shoot foxes because will cause dispersal.

DH: Define a good 10-20 km radius sampling area and focus on this re: fox feces. Focus on port and on road.

HW: Wants a 50km large area.

DH: If it's doable. Then it's no longer a 'focus'. If focus on 20km and intensively sample, and then see EM on the edge of the area, you already know that you no longer have a small focus.

HW: Best then to focus samples on 10-20 km outside of epicenter?

PD: Better to include the positive focus as well, because need some positive controls (if only focus on 10-20km outside of focus, don't know if you have false negatives if you find nothing. Need internal positive control).

HW: agreed.

HU: Nice to know how prevalent EM is in rodent population of positive area as well.

GO: Will have lots of traps out there, so will get good rodent coverage.

EÅ: Whatever we do, need to explain that with this multifaceted approach, we may eliminate EM, so it's worthwhile (even if we can't prove which method lead to the eradication).

HW: How much distance between positive samples is it worth to still bait?

DH: Need a cost analysis

HW: Need to agree on how large an area outside of the positive fox area is needed to be sampled to make sure it's not spreading. Is 10km enough? How big should the baiting area be? Need to include both the positive area plus a negative rim/zone. E.g. 10km of positive area, plus 10 km of negative area?

EÅ: Use fox home range to determine this size.

HW: HR for foxes is only 5 km2, so would be well within positive defined area.

GO: Also remember that this is an excellent fox habitat, so we're causing a source-sink artificially. By taking out foxes from the positive area, you'll be drawing in new foxes to the areas. There is for e.g. a nature reserve to the south where no foxes are hunted. This area could be producing lots of foxes that move into the positive area.

DH: Use three mean HR sizes as a start of your positive area, then calculate baiting area size and look at economics re: if its affordable.

EÅ: Start small, see if it's working, then expand baiting and surveillance.

CK: Has to leave, interested in ecology and would be very interested in doing something comparable in Denmark. Keep him posted.

HW: Have agreed that we need to do primary sampling in the whole area (not just in rim).

PD: We assume that there are more infected foxes. If none found, job is done?! Start small (within small area) and focus/target efforts. Then go from there. Not real research (because no control), but if Sweden does this intervention and never finds another positive, no one will be upset! Alternately- if you find other positive sites in next few weeks/months, then have to re-evaluate your strategy. Ok because by starting small, didn't use a huge amount of resources either.

DH: Really important to have an adaptive strategy. Start small-scale, then will have time to make a better decision on final baiting range size.

HW: Would like to include a probable source of EM introduction in the baiting/sampling area. How large then to make sure we include a possible introduction site? Don't want to have the area so small so that no possible introduction points are included.

DC: Point out possible introduction points, and include those too for fecal sampling. If introduction along coastal areas, infection must have somehow moved to where it was found. Ask fox people where foxes would migrate from/to?

HW: Grimsö researcher working on fox migration. Can ask him to work in this positive area, too.

Does it seem reasonable to bait in middle of nowhere?

DH: Yes- as a start. Then adapt strategy/baiting area as you get more data.

EÅ: Still 3-4 more weeks of sample analysis of foxes from this county. Makes sense psychologically to start baiting at positive epicenter.

DH: Then you will have time to develop and establish the method, get the experience on how to deal with samples and data. Then can expand. Question on distance between baits. 50 baits per km2 (Also said that 30 baits/km^2 might be enough since 50 was calculated to match the high fox densities of the Swiss investigated areas). Like to have minimum 50m between baits. Use a grid to evenly distribute baits in an area. Within each grid cell, target areas attractive to foxes. If big area, need to use airplane. GPS of where each bait is.

EÅ/GO: Could engage hunters to do some of this field work.

GO: if know where fox dens are present, can saturate bait around fox dens. Can also target sampling around here.

EÅ: When small focus, can start with manual baiting.

DH: If manually put out bait, can also collect fox feces at same time.

HW: Concern that feces collection wouldn't be consistent from area to area?

DH: Look for areas favorable for foxes. Concentrate baits and fecal collection here.

PD: Be aware that if you collect feces from a single place, can be collecting from the same fox. Not a true prevalence, but more of a pressure estimate.

DH: We have some general rules re: feces collection, but hard to define. For example, we don't take 5 feces all from same spot, but will take another scat sample from 20m away… Can try to have rules to help minimize sampling from same animal.

GO: But here we should take all feces away just to remove infection pressure/contamination. (Don't need to test all?)

DC: If take all feces from the same site in one bag, then you minimize contamination of environment. Only need to analyze one sample from the site.

HW: If baiting area is 10km radius, should include positive epicenter and will include negative border.

DH: And consider sampling potential introduction sites (roads, coast, dog areas).

GO: Have a dance hall in the middle of nowhere. People/foreign tourists come with dogs possibly.

HW: If we don't go for baiting, what should we do? Probably increased sampling/surveillance? Need to make recommendations. What recommendations are needed to give? Government wants report before summer when people out in woods. Need to calculate what each strategy (baiting/no baiting) would cost. Comments?

DH: Inform that risk is very low right now. Can't predict the future, but right now, don't need to scare the people.

DC: For the average person, either there's a risk or not. Hard to get across the concept of minimal risk.

UC: After 2011, should we continue surveillance in intervals, rather than continuously? i.e. monitor every 5 yrs?

PD: In Switzerland, we don't do surveillance anymore. Know we have the problem, too expensive to monitor. Don't bother keeping track of it anymore. However, Germans have done work on surveillance/monitoring and generated fantastic data. (Had leftover capacity from rabies days). Here- depends on what Swedish people want. Need to inform public how rare it is here now. Try not to make too big a deal about it.

HW: If apply recommendations from Germany and Switzerland to Sweden, it would be a big issue/change. Do recommendations change from high to low prevalence areas?

PD: Don't even have official recommendations in Switzerland. No national recommendation.

GO: Swedes are naïve to the risk of EM. Typical Swedish summer image is a child eating freshly picked forest berries. So either there is a risk or there is no risk for Swedish people. Devasting to authorities if they say there is no risk, then a human case pops up a year later.

HW: As a child in Switzerland/Germany, do they behave differently than Swedish kids?

PD: NewZealand in 70s did experiments with dogs and sheep and echinococcus hypothesized that flies could carry eggs between places. Someone then asked what happens if flies on feces land on berries and then contaminate the berries? This thought was then perpetuated (without evidence?)

HW: Anything that brings a human in closer contact with earth/soil/potentially contaminated habitat may be the risk factor.

PD: Not just the berries then, but the activity of being in the contaminated habitat, that is the risk factor. Once a year, we have a case of a kid eating fox feces. Mother grabs the feces and sends it for testing. Have found EM in one of these cases, but decided not to treat the child.

Followed the case up, and child still has no infection (it was deemed that treatment for child was too detrimental).

DH: In Switzerland, many are aware that EM risk is low. Most can distinguish between high and low risk.

PD: Risk of Borrelia/TBE much higher and people can differentiate between these risks and risk of EM. But can also do something active against Borrelia/TBE (vaccination, tick checks)

DC: So what's the active thing one can do to avoid risk of EM?

EÅ: Wash hands after being outdoors? What about in picnic situation? Should we try to get rid of EM if we can? In Sweden, maybe it's worth it trying to eradicate it? There is a huge diff between no risk and minimal risk.

PD: So far, you only have a single event, and in this place, you can show that you did something active. With data that you have now, you have a localized event, and no indication that widespread action is needed. I still hope that Sweden is like Denmark i.e. just a single event. Maybe there's something about Scandinavia that makes it parasite-unfriendly. With Sweden's high medical system, if there's a patient with EM, it should be detected. There are rare atypical human liver EM cases that are misdiagnosed, even by experts. (Have been 3-4 documented cases in Switzerland or in Germany). Need immunodiagnosis to catch every single case.

DC: In Sweden, approximately 50 cases of E. granulosus in humans in last decade, judged to be imported.

HW: Are there any countries with official EM recommendations?

PD: Probably France. Combes (AN comment- didn't get the name) in France could tell you.

HW: If Sweden chooses no baiting, would repeated surveillance be a requirement?

DC: Requirement for further data in Sweden will always be present.

HW: Probably don't have to recommend changes of childrens' behaviour in woods for EM-free parts of Sweden. But have to make recommendations for kids in affected parts.

DH: Be careful not to focus just on kids, because give false impressions that they're more susceptible.

HW: Children just one example of recommendations. Would probably just keep recommendations localized to positive area in Sweden?

PD: In Switzerland, we work with the medical office (not up to us/ the veterinarians, to take responsibility to inform the public). In Switzerland, it doesn't fall on veterinarians shoulders. It's up to the medical officers to inform the people and to decide how EM risk management is implemented.

DC: Same situation in Sweden.

PD: One recommendation that is highlighted in Switzerland is that of contaminated shoes. Shoes are not taken indoors. The shoe is the most contaminated object. Much higher risk to touch shoes than pick berries. Therefore they now work on education to make sure shoe hygiene is followed. (Leave dirty garden shoes outside). If you go out in woods, be aware of your shoes/boots (more important than the berries you pick)

GO: EM now only in woods in Sweden. But that doesn't mean we won't get it in more urban areas. We have urban foxes in Sweden, but don't have boot recommendation here in Sweden. We are totally naïve on this front.

UC: Unified that if we should do something, we should start this year, before this summer.

HW: Foxes start dispersing in Oct/Nov, so if we do anything, need to do it for the summer.

DH: If decide not to bait, need to explain why you decided not to.

HW: Biotope modeling. Need to know more about rodent populations

PD: Has been a lot of work done on rodents in France, then Germany did some work, and it was totally different from the France situation. (So be careful applying results across areas)

GO: That outcome should be expected. We can use older data as a starting point, but then need to look at our own biome.

PD: Lots of work done, but a lot of it only in theses (not in wider literature). PD can help get access to this unpublished data. So you don't repeat what already has been done.

GO: Also need to be aware that what's a suitable habitat in central Europe doesn't mean it's suitable here.

HW: Suggests this be taken up with EMIDA group.

PD: Yes, And the group is open for all.

HW: Suggests GO takes up this question and follows up on this rodent issue.

HW: Looked at age distribution of EM infected people and wanted to know why 66 yr olds get disease? Like TB, like herpes infection.

PD: Age distribution of AE in humans is much discussed. People use 5-15 years as incubation period, but very hard to definitively determine this. For many chronic diseases, they are often detected after 50 yrs of age, because people get more regular medical care after age 50 yrs. Contact with medical personnel in general increases after 50, so the system will pick up more. Also- AE is a chronic disease and is probably immunologically influenced to some extent. Some immune modulation is involved in the human host. Grows and grows, and then suddenly explodes.

DH. If EM age distribution is just related to increased health care after age 50, we would expect cases in people over 50 to be detected in earlier stages than in younger cohorts. Doesn't think this is the case.

PD: 20% have small lesion- best prognosis. Doesn't seem to be age-related. (AN comment- I missed this comment- it may be misquoted)

HW: Does infection occur in young people, then develops at different rates?

PD: Not logical that people at 35 have more contact/are more susceptible to EM.

HW: Outlines different tests for surveillance (SCT, SSCT, CoA, CoPCRPool, CoEgg PCR) and costs in SEK for different tests. CoPCRPool cheapest (550). Used a design prevalence of 0.1% (2 positive in 2000 samples). Want to find more positive cases. What is test that should be used to be as cheap as possible?

Cost per 1 positive finding (i.e. 1000 samples analysed, taking into account specificity)

SCT- 2.293 million(100%spec)

SSCT- 1.934 million (100% spec)

Cheapest = CoPCR Pool (like the Norwegians do) = 1.3 million per positive test

PD can't believe the prices for testing. Seem very inflated. But HW says this is what it costs/SVA takes.

PD: Coproantigen= 5-10euro needed per test. Can't calculate PCR costs separately in his lab because they use the sediment from CoproAntigen for next PCR step. (more efficient). Can combine coproAntigen and egg isolation PCR to be cheaper (55-60 euros). Use 2g feces ideally for these tests.

GO: For field work, could you use an anal swab from foxes for PCR?

DC: Not enough material. Need at least 1 g (PD likes 2g).

GO: Could you collect feces from hunted foxes 1-2g?

PD: Unfortunately, lots of shot foxes have no feces in rectum.

EÅ: Would be really nice to get feces from shot foxes instead of whole fox.

HW: Intensive surveillance is difficult to fund based on costs per positive sample presented above.

PD: If egg isolation is not much more expensive than coproantigen test, maybe go with that test? Keep some material so that if a sample is positive, you can go back and see which one of the pool was positive.

DC: yes. Fox feces is 2-3g, so perfect size. Here at SVA use 3g from a single (not pooled) sample. Warns that sensitivity of pooled samples can be dangerously diluted.

HW: Wrapping up the meeting. If Sweden baits, asks DH if he's interested in collaborating? Yes, as much as he is able to. UC believes that it would be very valuable to bring DH back if they go ahead with baiting.

End.