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‣ Løbe nr. I1RG9 Fare for sygdomme ved fodring med insekter & regnorme

Baggrund, relevans og perspektiv

‣ Insects are hosts to many insect-specific viruses (e.g. baculoviruses) (Maciel-Vergara & Ros, 2017), which are unlikely to represent a threat to mammalian, avian or aquatic species as most of them do not replicate in the cells of these other species. However, in addition, insects can be infected by certain viruses, termed arboviruses, that are also able to infect mammalian species. The arboviruses (over 500 known examples) replicate within the insect host and thus amplify the amount of virus that can then be passed to mammalian (or other) hosts, to initiate infection and replication, when these insects then feed on them. Important examples include mosquito-borne viruses such as West Nile virus and Usutu virus (both flaviviruses), midge-borne viruses such as bluetongue virus (a reovirus) and Schmallenberg virus (an orthobunyavirus) plus the soft tick-borne African swine fever virus (ASFV); this is the only known arbovirus with a DNA genome. The replication of the virus in the insect host clearly enhances the possibility of virus transmission compared to instances of insects acting as a “mechanical vector” when no virus replication occurs within the insect but they function essentially as “flying syringes”, transferring virus containing blood from one mammalian host to another.

Insects can ingest viruses from mammalian, avian or aquatic hosts, e.g. from wild boar, fish or birds, and then potentially the larvae or adult insects could be eaten by another susceptible host. The duration of survival of the infectious virus within the insects then becomes an important issue. Similarly, if feed, potentially inadvertently contaminated with virus (e.g. with avian influenza virus (AIV)) is fed to insects and infectious virus is still present within the insects at the time of feeding to a susceptible host (e.g. poultry or pigs) then virus transmission could occur. The level of virus taken up by insects during feeding on contaminated feed and the dose required for infection of the animal by oral ingestion are clearly issues here. Some viruses are not readily transmitted by the oral route and thus even if the virus is present within feed the risk of viral transmission is very low.

Even between viruses of the same virus family, there can be marked differences in the ability of the viruses to replicate in both insects and mammalian species. For example, among the flaviviruses, some well-known arboviruses exist, e.g. Yellow fever virus, West Nile fever virus and Usutu virus, while other members of this family only replicate within mammalian hosts, e.g. the pestiviruses (such as classical swine fever virus) and the hepaciviruses (e.g. hepatitis C virus). It

is apparent that the flaviviruses that replicate in insects have a genome that is translated (to proteins) in a cap-dependent manner (a mechanism that is ubiquitous amongst eukaryotic cells) whereas the viruses that only replicate in mammalian hosts use an internal ribosome entry site (IRES), within the 5'-untranslated region, to direct cap-independent translation initiation. Thus, the host tropism reflects the molecular features and properties of the viral genome. It cannot be expected that the viruses that contain an IRES, which only functions in mammalian cells, will easily adapt to grow in insect cells. This host restriction probably limits the ability of certain other viruses, e.g. foot-and-mouth disease virus (a picornavirus), with a different type of IRES that functions in mammalian cells, from growing in insect cells.

A study on risks related to consumption of mealworms or crickets concluded that there is a low risk of prions and foodborne viruses in insects reared for human consumption, and that such cases have not yet been described (Cappelli et al., 2020). A summary review of biological risks in edible insects likewise concludes that there is barely any data on this topic yet for viruses, while for prions, it will be a matter of controlling the substrates of the insects to minimize the risk of introducing prions into the insect production (Vandeweyer et al., 2021).
In a risk assessment from EFSA, the risk of transmission of viruses is judged to be negligible except for the cases of arboviruses, and for stable viruses capable of mechanical transfer via insects. Here it is recommended to perform proper treatment of substrates (e.g. heat-treatment of manure) and effective processing of the insects (Hardy et al., 2015).

Metode, data m.m.

Clarification of key issues between FVST and DK-VET regarding this task
It has been discussed and agreed between FVST and KU-VET that this task does not include insects and earthworms taken up by the animals in the field, as this is not a part of the topic for this task that is related to regulation of use of live insects for feed.
In addition, it is mentioned in "the bestilling" that "mikrobiologi" should not be included, which should be understood as meaning that only viruses and TSEs are included in this report.
Limitations for the review
As mentioned in the introduction above, experimental studies that specifically investigate transmission of animal viruses using insects or earthworms as feed are scarce or non-existent.
Therefore, this review has focused on firstly, collecting virus related studies for the specifically listed insect species in the task describing various factors such as vector competence, virus persistence in the insect and secondly, to describe the specifically listed viruses in the task with regards to transmission, persistence and described associations with insects.
Conclusions from such studies do not necessarily translate into actual transmission of animal viruses using insects as feed, but they indicate potential risks.
In the discussion, relevant viruses and insects for the requested groups of domestic animals have been initially evaluated for these potential risks.

Resultater

Specific insects and described associations with viruses
Black Soldier Fly (*Hermetia illucens*) larvae are currently produced on the largest scale in Europe. They are fed on a variety of agricultural waste products and in some parts of the world they can even be fed on manure (Olesen et al., 2021). Larvae, pupae and pre-pupae are used (Hardy et al., 2015).
There are currently no described viruses specific for black soldier flies and they are generally thought of as highly resistant to infections and diseases (Joosten et al., 2020).

House flies (*Musca domestica*) are efficient converters of agricultural waste with a short lifecycle. While not quite as popular now as *H. illucens, M. domestica* could become more important in the future. However, another very important feature of this organism is that it is present in any insect production system as one of the main pests. It is inadvertently reared along with *H. illucens*, as it is able to infiltrate the production system and benefit from the ideal conditions for growth (Olesen et al., 2021). The larvae are reared for feed for food-producing animals (fish, chicken, pigs), either fresh or as a dried and powdered protein supplement (Hardy et al., 2015).
House flies have a specific virus (*Musca domestica* salivary gland hytrosavirus), which causes enlarged salivary glands and reduced fertility. House flies have been investigated as potential mechanical vectors for many viruses including AIV (Nielsen et al., 2011; Salamatian et al., 2020; Wanaratana et al., 2011, 2013), PRRSV (Otake et al., 2003, 2004; Pitkin et al., 2009; Schurrer et al., 2005), PEDV (Masiuk et al., 2018), TGEV (Gough, 1983), Aujeszky's disease (Zimmerman, 1989), ASFV (Fila & Woźniakowski, 2020; Turčinavičienė et al., 2021), lumpy skin disease virus (Sprygin et al., 2018, 2019), Rift valley fever virus (Turell et al., 2010), Newcastle disease virus (Barin et al., 2010; Chakrabarti et al., 2008; Watson et al., 2007) and SARS-CoV-2 (Balaraman et al., 2021; Soltani et al., 2021).

Mealworms (*Tenebrio molitor*) are currently produced on the largest scale in Europe. They are fed on a variety of agricultural waste products and in some parts of the world they can be fed on manure (Olesen et al., 2021). Mealworms are easy to rear and are produced for live pet feed in many countries and in some countries also for human consumption. The larval stage is used (Hardy et al., 2015).
No studies were found describing vertebrate animal viruses in mealworms, but a densovirus of crustaceans was shown to be able to replicate in the cells of mealworms and to a larger extent in house crickets (la Fauce & Owens, 2008)

Lesser mealworms (*Alphitobius diaperinus*) are reared in considerable volumes in Europe, they have a very similar lifecycle and diet to *T. molitor*. However, they are currently not quite as popular (Olesen et al., 2021). Mealworms are easy to rear and are produced for live pet feed in many countries and in some countries also for human consumption. The larvae are used (Hardy et al., 2015).
Darkling beetles (the adult stage) are pests in poultry farms and have been investigated in relation to transmission of several poultry related pathogens e.g. bacteria, including Salmonella, Campylobacter and also Histomonas protozoan parasites. Many viruses have been investigated as well including ILTV (infectious laryngotracheitis virus)(Ou et al., 2012), IBDV (infectious bursal disease virus) (Mcallister et al., 1995), turkey coronavirus (Watson et al., 2000), fowl pox and Newcastle disease (de Las Casas et al., 1976)

House crickets (*Acheta domesticus*) are becoming increasingly popular, and while some stumbling blocks remain (rearing design and sustainable diet), these crickets have the potential to become a very nutritious and important source of protein for livestock (Olesen et al., 2021). They are farmed for live pet feed in many countries, also in Europe. In the Netherlands and Denmark they are being farmed to be marketed for human consumption. The adult stage is used (Hardy et al., 2015).
House crickets are hosts to *Acheta domesticus* densovirus (a parvovirus) that causes great problems in commercial rearing (la Fauce & Owens, 2008; Szelei et al., 2011; Weissman et al., 2012). As mentioned above, a densovirus of crustaceans has been shown to replicate in house crickets (la Fauce & Owens, 2008). The virome has been investigated for house crickets (de Miranda, Granberg, Low, et al., 2021; de Miranda, Granberg, Onorati, et al., 2021)

Tropical house cricket (*Gryllodes sigillatus*) are not produced in any considerable quantities in Europe, either because they are too hard to produce, or because no-one has yet thought of it.

Information and relevance for feed and food is lacking (Olesen et al., 2021). They are farmed for live pet feed, where the adult stage is used (Hardy et al., 2015).
No studies were found describing viruses in house crickets except for that the *Acheta domesticus* densovirus is not able to replicate in the tropical house cricket (Weissman et al., 2012)

Jamaican field cricket (*Gryllus assimilis*) are not produced in any considerable quantities in Europe, either because they are too hard to produce, or because no-one has yet thought of it. Information and relevance for feed and food is lacking (Olesen et al., 2021). The adult stage is used (Hardy et al., 2015).
No studies were found describing viruses in field crickets except for that the *Acheta domesticus* densovirus is not able to replicate in the tropical house cricket (Szelei et al., 2011)

Other relevant insects in Denmark:
Migratory locust *(Locusta migratoria)* - some companies have started producing *Locusta migratoria,* and EFSA has authorized their use this year. Hence, the production numbers might increase (Olesen et al., 2021). Worldwide, grasshoppers are consumed from wild collection. Some tropical countries hesitate to promote their farming due to crop pest risks if released. The adult stage is used (Hardy et al., 2015).
No studies were found describing viruses in migratory locusts except that an iridovirus of crickets (Kleespies et al., 1999) and entomopoxviruses of locusts were suggested for (migratory locust) pest control (Strett, 1997)

Greater wax moth (*Galleria melonella)* are produced in quite large quantities and are widely used as alternative non-mammalian models for the study of pathogens, even though they are not really popular for food or feed. The larvae can be acquired in small volumes from worm farms, pet stores, or other independent suppliers commonly found in the United States and parts of Europe, so the additional step for their use as food and feed is not a massive stretch (Olesen et al., 2021).
These moths are important pests in honeybee production, and they have been shown to carry honeybee viruses (Traiyasut et al., 2016). They are susceptible to Nodamura virus which is able to infect fish, mammals (mice) and insects and which performs virus replication in complexes that are targeted to mitochondria (Gant et al., 2014).

Silkworms (*Bombyx mori*) are very widely produced in Asia for silk production and the leftover larval and cocoon stages are used for food, but whether it will ever make it to the feed market in Europe is uncertain (Olesen et al., 2021). They are also used extensively in molecular biology, as e.g. model organisms and for protein expression using a baculovirus system. Baculoviruses do not replicate within mammalian cell systems, an important safety feature of this system. An important natural infection of silkworms is the nucleopolyhedrosis densovirus. Silkworms are also permissible to *Spodoptera frugiperda* rhabdovirus, which, although related to rabies virus, is not able to replicate in human or other animal cell lines (Schroeder et al., 2019). No direct connections to vertebrate animal viruses were found.

Earthworms are naturally used for feed by free-range animals (poultry and pigs), and some hobby-farmers grow their own earthworms for animal feed-supplement. Commercial rearing of earthworms is under consideration for poultry and fish farming (Parolini et al., 2020).
Denmark has 22 species of earthworms that all belong to the family *Lumbricidae* (https://allearter-databasen.dk/?perma=518a47cc166d9). The 5 most common species are: Common earthworm (*Lumbricus terrestris* Da. stor regnorm), the grey worm (*Aporrectodea caliginosa* Da. grå orm), blackhead worm (*Aporrectodea longa* Da. lang orm), the rosy-tipped worm (*Aporrectodea rosea* Da. rosa orm) and the green worm (*Allolobophora chlorotica* Da. grøn orm). No studies of viruses in these five earthworm species were found.

Specific viruses, transmission, persistence and described associations with insects

African Swine Fever Virus (ASFV)
As indicated previously, (Olesen et al., 2021): African swine fever is a serious contagious livestock disease present in Europe. The virus has been shown to be very stable and with long-term maintenance of infectivity within certain materials. Viruses replicate both in pigs and in invertebrate vectors (soft ticks). The virus is transmitted very efficiently via meat and meat products and this route of transmission is well documented.

Classical Swine Fever Virus (CSFV)
As stated previously, (Olesen et al., 2021): Classical swine fever is a serious exotic contagious disease of swine (domestic and wild). Import of pig products from endemic areas could result in virus presence in food or catering waste. Transmission of CSFV via swill feeding to pigs is well-documented.

Avian Influenza virus (AIV)
As stated previously, (Olesen et al., 2021): Avian influenza virus (Influenza A) is a contagious zoonotic livestock disease that is present in wild birds and can be introduced by migratory birds. Poultry are particularly vulnerable to infection and disease, but pigs and humans can also be infected, thereby risking reassortment and the emergence of new variants with potential pandemic potential. Wild birds can transmit the virus via bird droppings e.g. to vegetables grown in fields and thus viruses may be present in food waste used as feed for insects. Once an avian influenza virus has entered a poultry flock, it can spread on the farm by both the fecal–oral route and aerosols, due to the close proximity of the birds. Fomites can be important in transmission, and flies may act as mechanical vectors. Transmission via meat and other biproducts from HPAI that causes systemic infection cannot be ruled out. HPAI (H5N1) has been detected in infected duck meat. There is no direct evidence that AI viruses can be transmitted to humans via the consumption of contaminated poultry products.

Foot-and-Mouth Disease Virus (FMDV)
As stated previously, (Olesen et al., 2021): Foot-and-mouth-disease virus is a serious exotic highly contagious livestock virus, which causes acute vesicular disease in all cloven hoofed animals. The virus is easily capable of airborne spread, and is therefore difficult to contain in outbreak situations. Fomite and animal products can carry infectious virus. Import of cloven-hoofed animal products from endemic FMDV areas could result in virus presence in food or catering waste. Airborne transmission and contact transmission are the most common routes of transmission, but transmission via offal to pigs is also well-documented.

SARS-CoV-2
As stated previously, (Olesen et al., 2021): SARS-CoV-2 is a newly emerged human pandemic zoonotic virus that (in addition to humans) easily infects mink. Aerosols, direct-contact, animal-to-human, and human-to-animal transmission represent major routes of transmission for SARS-CoV-2. There have been concerns raised that the virus can be transmitted via contaminated meat.
It was recently concluded that the risk of SARS-CoV-2 transfer via edible insects is extremely low, due to the inability of coronaviruses to replicate in insects and that the virus would be destroyed (or inactivated) through processing of the insects after production (Dicke et al., 2020).

Hepatitis E (HEV)
As stated previously, (Olesen et al., 2021): Hepatitis E virus (HEV) is primarily a human pathogenic virus with 70 million newly infected people per year in the world. Genotypes 1 and 2 exclusively affect humans but there are 8 genotypes in total. Infection among humans occurs via the fecal-oral route through fecal contamination of water and food. Most cases reported in Denmark occur after travel abroad. There is also a reservoir in pigs of genotypes 3 and 4 HEV, and humans can

be infected via undercooked meat (liver). There is a risk of transmission of HEV to insects if material contaminated with human feces or e.g. non-heat-treated pork is used as insect feed.
The presence of hepatitis E virus (along with hepatitis A virus and norovirus genogroup II, two other common foodborne viruses) was investigated in commercially reared raw mealworms and cricket species from Belgium and the Netherlands, and was not detected (Vandeweyer et al., 2020). However, it is not known whether changing the feed substrate for the insects would affect this finding.

Transmissible Spongiform Encephalopathy (TSE)
As stated previously, (Olesen et al., 2021): Transmissible spongiform encephalopathy is a palette of fatal diseases in several animal species including humans arising from the accumulation of the misfolded prion protein. Prions are extremely stable, can remain infectious for a long time and are transmitted orally either directly (by ingestion of misfolded prion protein from infected host) or indirectly via an infected environment, plants and insects. Prions have so far not been found to have originated in insects. Misfolded prion proteins from BSE infected cattle (nervous tissue) can be transmitted via food to humans. Variant-CJD transmits after ingestion of infected beef.
In the risk assessment from EFSA on the biological hazards of insects as food and feed it is stated: "*Cellular prion proteins are not naturally expressed in insects. Therefore, no relevant risks exist in relation to insect-specific prions. Similarly, mammalian prions cannot replicate in insects, and therefore insects are not considered to be possible biological vectors and amplifiers of prions. Various studies suggested the possible role of insects as mechanical vectors of infectious prions. Insects farmed on a substrate or in an environment in which infectious prions are present could act as mechanical vectors of infection and represent a potential risk of transmission of prion diseases through food and feed. The total prion infectivity carried by insects would depend on the amount of infectivity present in the substrate used and can only be equal to or smaller than this*" (Hardy et al., 2015).

Other relevant diseases could be considered, see for example Table 2 and Table 3 in the response to a previous task (Olesen et al., 2021).
For aquaculture, these other relevant diseases could for a start be those that are listed in "Listebekendtgørelsen":

- Epizootic haematopoietic necrosis virus (Da. epizootisk hæmatopoietisk nekrose) (EHN)
- Spring viraemia of carp (Da. forårsviræmi hos karper) (SVC)
- Salmonid alphavirus (Da. Salmonid alphavirus) (SAV)
- Infectious hematopoietic necrosis virus (Da. infektiøs hæmatopoietisk nekrose) (IHN)
- Infectious salmon anaemia (Da. infektion med infektiøs lakseanæmi-virus) (ISA)
- Taura syndrome virus (Da. infektion med Taura-syndrom-virus) (TS)
- Viral haemorrhagic septicaemia (Da. viral hæmoragisk septikæmi /Egtved syge) (VHS)
- White spot syndrome virus (Da. infektion med white spot syndrome-virus) (WSS)
- Yellow head virus (Da. infektion med yellow head disease-virus) (YHD)
- Infectious pancreatic necrosis virus (Da. infektiøs pankreasnekrose) (IPN)
- Koi herpesvirus (Da. koi-herpesvirus-sygdom) (KHV)

Links to insects were discovered for the following of these listed viruses:

Mosquito cells were permissible to IHN virus and produced virus titres comparable to those seen in fish cell culture. The produced virus was found to be pathogenic to rainbow trout, and it was concluded that mosquitos could be possible vectors for IHN (Scott et al., 1980). Furthermore, infectious IHN virus was isolated from mayfly insects (Shors & Winston, 1989). It has also been confirmed that insects can carry IHN virus, when they are migrating upstream, and that freshwater leeches and copepods can contain infectious IHN (OIE, 2021).

A *Drosophilla melanogaster* cell line was investigated for permissiveness to infection with three fish rhabdoviruses, and two of them (SVC and pike fry rhabdovirus) were found to replicate in these cells (Busserau et al., 1975).

An H5 insect cell line (commonly used with the baculovirus system) was found to be permissible to infection with VHS virus, producing characteristic syncytia as a sign of virus replication. The propagated virus from the insect cells was still pathogenic to trout fish after two cell culture passages (Lorenzen & Olesen, 1995).

The shrimp YHD virus could be cultured in immortal mosquito cell lines, but showed attenuation after serial passaging (Gangnonngiw et al., 2010).

Common biological vectors of fish diseases (e.g. viruses) include their crustacean parasites e.g. fish lice, which are able to transfer SVC and carp pox (Overstreet et al., 2009).
Crustaceans are related to, but different from, insects and they both belong to the *Arthropoda* phylum of the animal kingdom. This relationship is exemplified by the fact that some shrimp parvoviruses are closely related to insect parvoviruses (Roekring et al., 2002), and that the shrimp pathogen Taura syndrome virus is closely related to the insect specific discistroviruses Cricket paralysis like viruses (Mari et al., 2002).

Diskussion

Relevant insects and viruses listed by groups of domestic animals

Horses

Horses could potentially become exposed to insect viruses through grazing via ingesting virus-containing insects. However, to our knowledge there are no described examples of this.
Arboviruses infecting horses use flying blood-sucking or biting insects or ticks as vectors. The risk of horses contracting a specific virus while grazing, is completely dependent on the geographic distribution of the virus and its arthropod vector.
None of the insect species listed in this task for live feed are biological vectors for these viruses.
Horses would not willingly consume live insects as feed, and the diet composition would be wrong.

Aquaculture

Relevant viruses for aquaculture listed in task:

1. Other relevant diseases (those that are listed in “Listebekendtgørelsen” for aquaculture)

In the few studies on aquaculture viruses and insects mentioned in the results section, it is apparent that some of these serious viruses are capable of replicating in insect cells. Therefore, it cannot be ruled out that there could be a risk associated with using insects as feed, especially if the substrate of the insects contains aquaculture derived material (and possibly virus).
We conferred with colleagues at DTU Aqua on this matter (Professors Niels Jørgen Olesen and Niels Lorenzen). They informed us that researchers in the Netherlands are working on this issue at present, and that DTU Aqua would be interested in conducting separate investigations on this too.

Relevant insects for aquaculture:
At present, extracts from black soldier fly larvae and housefly larvae are commonly used. However, for feeding with live insects, the size of the insects limits which fish and other aquaculture animals would be able to eat them.

Pigs
Relevant viruses for pigs listed in task:
1. African Swine Fever Virus (ASFV)
2. Classical Swine Fever Virus (CSFV)
3. Foot-and-Mouth Disease Virus (FMDV)
4. Hepatitis E Virus(HEV)
5. Avian Influenza virus (AIV)
6. Transmissible Spongiform Encephalopathy (TSE)
7. Other relevant diseases

Pigs can become infected with ASFV through ingestion of stable flies containing this virus. This form of mechanical vectoring is also theoretically possible for the other listed diseases that can be transmitted orally. Therefore, it is important to control the substrate provided to the feed-producing insects, to make sure that such contaminants are limited.

An exception would be hepatitis E virus, which is already present to a high degree in domestic pigs. Ingestion of insects containing hepatitis E virus, would probably not contribute further to the high prevalence that we see already in pigs in Denmark.
Relevant insects for live feeding of pigs could be black soldier fly larvae, mealworms, lesser mealworms and earthworms (reared or dug up from the field by pigs on pasture). Housefly larvae would not be recommended due to their ability to transfer pathogens.

Broilers (*Gallus gallus)* and turkeys
Relevant viruses for poultry (broilers and turkeys) listed in task:
1. Avian Influenza virus (AIV)
2. Transmissible Spongiform Encephalopathy (TSE)
3. Other relevant diseases

As discussed above, it is important to control the substrate provided to the feed-producing insects, in order to ensure that such virus or prion contaminants are limited.

Relevant insects for live feeding of broilers and turkeys could be black soldier fly larvae, mealworms and earthworms (reared or dug up from the field by free-range birds). Housefly larvae and lesser mealworms would not be recommended due to their ability to transfer pathogens.

Other poultry
Relevant viruses for other poultry than broilers and turkeys listed in task:
1. Avian Influenza virus (AIV)
2. Transmissible Spongiform Encephalopathy (TSE)
3. Other relevant diseases

As above, it is important to control the substrate provided to the feed-producing insectsin order to ensure that such virus or prion contaminants are limited.
Relevant insects for live feeding of other poultry could be black soldier fly larvae, mealworms and earthworms (reared or dug up from the field by free-range birds).Housefly larvae and lesser mealworms would not be recommended due to their ability to transfer pathogens.

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