

Role of Insects in Spread of Foodborne Pathogens

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Contributor: Remigiusz Gałęcki, Tadeusz Bakula, Janusz Gołaszewski

Insects play a key role in European agroecosystems. Insects provide important ecosystem services and make a significant contribution to the food chain, sustainable agriculture, the farm-to-fork (F2F) strategy, and the European Green Deal. Edible insects are regarded as a sustainable alternative to livestock.

Keywords: foodborne pathogens ; biosecurity ; food chain

1. Introduction

Insects (class Insecta) are ubiquitous in the world ^[1], and they come into direct contact with humans ^{[2][3]}. Social attitudes toward insects vary. In some countries, insects are regarded as ectoparasites and pests. However, in some cultures and ethnic groups, insects, as a source of protein and other nutrients, have been a part of the human and livestock diet for many centuries ^[4]. Many insect species are also used in traditional medicine around the world ^[5]. Insects are used in production of vaccines and protein preparations ^[6]. In 2004, extracts from *Lucilia sericata* larvae became the first insect-based treatment for chronic wounds that has been approved for use in the United States ^[7]. The venom of the Samsun ant (*Pseudomyrmex* sp.) has numerous medicinal properties. This powerful antioxidant has been shown to reduce inflammation, relieve pain, inhibit tumor growth, protect the liver, and aid hepatitis treatment ^{[8][9]}. Insects are also farmed animals ^[10]. Honey bees (*Apis mellifera*) have been exploited for honey for many millennia, whereas domestic silk moths (*Bombyx mori*) and Chinese oak silk moths (*Antheraea pernyi*) have long been reared for silk. Insects are also in human and animal diets.

Entomophagy, namely the practice of eating insects, continues to attract the interest of researchers, ecologists, and consumers as a potential solution to feeding the world's growing population in the coming decades ^{[11][12]}. In recent years, insects have emerged as one of the most innovative substrates in human and animal nutrition ^{[13][14]}. According to many scientists, edible insects are a major milestone in efforts aiming to diversify protein sources and guarantee global food security ^[15]. Edible insects are most widely consumed in subtropical and tropical regions, but entomophagy is not highly popular in Western culture ^[11]. Global insect consumption is difficult to estimate, but, according to the literature, around 2000 insect species are consumed in more than 80 countries ^{[16][17]}. The most widely consumed insects belong to the orders Coleoptera (31% of global consumption), Diptera (2%), Hemiptera (10%), Hymenoptera (14%), Isoptera (3%), Lepidoptera (18%), Odonata (3%), and Orthoptera (13%) ^[18]. Around 1500 species of wild and farmed edible insects are eaten in Africa ^[19]. Nearly 96 tons of edible insects are consumed in the Democratic Republic of Congo each year, and, in Kinshasa alone, an average family consumes around 300 caterpillars per week ^[20]. Latin America is the second largest market of edible insects, and entomophagy is most popular in Brazil, Ecuador, Colombia, Mexico, Peru, and Venezuela ^[21]. The Asian insect market is highly innovative ^[22]. In Asia, insects are not only popular substrates in food and feed production but are also used in the pharmaceutical industry ^[22]. Until recently, edible insects had not been regarded as a major food source in Europe. A breakthrough came on 20 December 2017, when a list of novel foods, including insects, was introduced by Commission Implementing Regulation (EU) 2017/2470 ^[23].

All arguments in favor and against entomophagy should be considered to promote introduction of long-term sustainable solutions on the European food market. Safety of edible insects should also be thoroughly analyzed before these products are authorized for human, companion animal, and livestock consumption. Numerous guidelines have been developed to ensure that edible insects are reared under safe conditions and can be safely used in food and feed production ^{[24][25][26]}. Despite the fact that most species of edible insects are harvested without proper biosecurity from the natural environment ^[27], farmed insects have to meet additional food safety standards and guidelines, including control of foodborne pathogens ^{[28][29][30]}. For this reason, microbiological safety of edible insects has to be thoroughly researched before they are approved for mass production. The optimal parameters for insect rearing, processing, and storage have already been described in the relevant regulations, but many edible insect species have not been tested for microbiological safety. Edible insects can be a source of biological hazards, including bacteria that cause foodborne diseases, and insect-based

foods can become contaminated in all stages of production, delivery, and consumption. Other biological risks associated with insect farming, such as use of organic side-streams and food wastes in insect nutrition, are often disregarded.

2. The Role of Insects in Spread of Pathogenic Microorganisms and Foodborne Pathogens

Edible insects are regarded as a safe dietary alternative in livestock production [31][32][33]. However, microbiological safety of insect-based foods intended for human consumption is still under debate [29][34]. EFSA outputs on safety evaluation of such products have confirmed safety of edible insect consumption under certain conditions of use [35]. Insect farming can contribute to decreasing prevalence and spread of selected contagious diseases, including foodborne diseases, by eliminating pathogen carriers/reservoirs from the food chain. Due to species specificity and the specific physiology of insects, most entomopathogens do not play a role in epidemiology of zoonoses and do not pose a threat to humans [36]. Arthropods' ability to transmit foodborne pathogens and vector-borne diseases has been widely researched in the context of food production and the One Health approach [37][38][39]. Edible insects are highly unlikely to act as disease vectors [36][40]. Industrially farmed insects are fed agri-food by-products and plant-based products; therefore, the risk of transmission of zoonotic pathogens is low. Entomopathogens cannot cross the species barrier and cause disease in mammals, which is why edible insects are safe to use in food and feed [36]. It is worth noting that, in some cultures, insects infected with pathogens are regarded as a culinary delicacy or as medicinal products [41][42].

There is no evidence to suggest that edible insects harboring bacterial and viral entomopathogens pose a threat to vertebrates [43][44][45]. However, similar to other foods of animal origin, insect-based foods can raise safety concerns because problems can arise after death of insects and during their processing [46]. Companies that rear and process insects must implement strict sanitary rules to ensure microbiological safety of the end product [10][47][48]. Dedicated processing operations are put into place to eliminate any foodborne pathogens. However, the substrate and end product can become infected during processing. To minimize risk, insect farms should abide by the same biosecurity standards that are applied in the conventional food sector [10][24]. Work surfaces should be disinfected, farm workers should maintain good personal hygiene, farm premises should be regularly cleaned, and safe food preparation and delivery practices should be observed [49]. In farms that have not implemented biosecurity measures, insects and insect-based foods can become contaminated with pathogenic microorganisms transmitted by personnel and pests [50][51]. Therefore, legal regulations, in particular veterinary supervision procedures, should be introduced to guarantee safety of insects as a novel food [52]. Similar to other food products, edible insects are sensitive to deviations from approved production or distribution standards [53][54]. The end product can become contaminated when the required parameters are not observed during acquisition of raw materials, processing (such as drying), transport, storage, and distribution. The associated risks are presented in **Table 1**. Edible insects as final products should be regularly monitored for presence foodborne pathogens to ensure their safe implementation in the farm-to-fork (F2F) strategy and the European food chain.

Table 1. Possible routes of contamination of edible insects and insect-based foods.

Stages of Contamination	Risks	Treatment	Reference
Substrate	<ul style="list-style-type: none">• (Crickets) Minimal impact of external microbiota.		
	<ul style="list-style-type: none">• (Crickets) Bacterial endospore counts in crickets fed a standard + farm weed (S + W) diet were significantly lower and thus promising and could reduce risks associated with ready-to-eat insects.		[55][56][57]
	<ul style="list-style-type: none">• Risk of contamination with <i>Salmonella</i> spp. and <i>Campylobacter</i> spp. increases if materials such as used paper egg cartons are utilized in insect rearing. This risk is higher if cartons had been in contact with poultry feces.		

Stages of Contamination	Risks	Treatment	Reference
Rearing	<ul style="list-style-type: none"> (Crickets) <i>Aspergillus flavus</i> strains with low mycotoxigenic potential were identified in reared crickets, which could point to presence of mycotoxins in edible crickets. 		[55]
Harvest	<ul style="list-style-type: none"> (Crickets) Starvation is not an effective method for reducing microbial loads in edible crickets. 	Gut emptying by starvation prior to killing could reduce the microbial load in the insect gut, but it could also decrease fat and energy content and profitability of production.	[58]
Processing	<ul style="list-style-type: none"> (Crickets) High microbial loads of TAC and Enterobacteriaceae were detected in edible crickets, indicating a high risk of rapid spoilage. (Crickets) Sporulating bacteria are a part of the cricket microbiome Food safety risks associated with viruses are very low. <i>Vibrio</i> spp., <i>Streptococcus</i> spp., <i>Staphylococcus</i> spp., <i>Clostridium</i> spp., and <i>Bacillus</i> spp. were identified in several studies on the microbiota of processed edible insects sold online. 	Thermal treatments, novel processing methods (i.e., high-pressure processing), and additional post-processing treatments (acidification, addition of food preservatives, modified atmosphere packaging, etc.) should be applied to extend crickets' shelf-life.	[55][59][60]
Transport		https://ipiff.org/wp-content/uploads/2019/12/IPIFF-Guide-on-Good-Hygiene-Practices.pdf (accessed on: 13.November.2022)	
Preparation	<ul style="list-style-type: none"> Dried mopane worms, termites, and stink bugs sold at the Thohoyandou market were characterized by low contamination with coliforms, <i>Escherichia coli</i>, <i>Staphylococcus aureus</i>, <i>Salmonella</i> spp., TPC, yeasts, and molds. 		[61]
Storage	<ul style="list-style-type: none"> (<i>T. molitor</i>, <i>Alphitobius diaperinus</i>, <i>Gryllus assimilis</i>, <i>Lo. Migratoria</i>) microbiological characteristics in different storage periods—safe for human consumption. 	Insects intended for long-term storage should be killed in boiling water, dried at 103 °C for 12 h, and hermetically packed.	[62]

Stages of Contamination	Risks	Treatment	Reference
Consumption	<ul style="list-style-type: none"> The nutritional value and the microbiological and toxicological profiles of insects are influenced by composition of organic side streams. The microbial risks associated with edible insects can be substantially reduced by observing good hygienic practices in rearing, handling, harvesting, processing, storage, and transport of insects and insect-based products. Several spoilage-causing microbes that can alter food quality, including <i>Lysinibacillus</i> sp. and <i>Bacillus subtilis</i>, have been detected in edible insects. 		[18][28][59] [63]
	<ul style="list-style-type: none"> Yeasts, including <i>Tetrapisispora</i> spp., <i>Candida</i> spp., <i>Pichia</i> spp., and <i>Debaryomyces</i> spp., and molds, including <i>Aspergillus</i> spp., <i>Alternaria</i> spp., <i>Cladosporium</i> spp., <i>Fusarium</i> spp., <i>Penicillium</i> spp., <i>Phycomycetes</i> spp., and <i>Wallemia</i> spp., are associated with the microbiota found on the body surface or in the gut of edible insects and may be harmful. 38 samples of deep-fried and spiced <i>Ach. Domesticus</i>, <i>Lo. Migratoria</i>, and <i>Omphisa fuscidentalis</i> tested negative for <i>Salmonella</i> spp., <i>Listeria monocytogenes</i>, <i>E. coli</i>, and <i>S aureus</i>, but dried and powdered insects, as well as pollen, contained <i>Bacillus cereus</i>, coliforms, <i>Serratia liquefaciens</i>, <i>Listeria ivanovii</i>, <i>Mucor</i> spp., <i>Aspergillus</i> spp., <i>Penicillium</i> spp., and <i>Cryptococcus neoformans</i>. 		
R&D	<ul style="list-style-type: none"> (Crickets) Further efforts are needed to identify food-borne pathogens in edible crickets and define possible bacterial quality reference values. 		[55]

Consumption of unprocessed insects may represent a significant risk factor. Insects can act as mechanical or biological vectors of pathogens [37], particularly critical priority pathogens in the food processing industry, including *Bacillus* spp., *Clostridium* spp., *E. coli*, *L. monocytogenes*, *Salmonella* spp., and *Staphylococcus* spp. [64][65][66][67]. Bacteriological hazards have been most widely investigated, but insects can also act as intermediate hosts or mechanical vectors for parasites in the natural environment [38]. Therefore, effective processing operations should be implemented and sanitary guidelines should be observed to minimize risk of contamination with foodborne pathogens [51].

Allergenicity of edible insects is yet another important safety concern. Similar to other food products, edible insects could pose certain risks to consumers with allergies. To date, 239 arthropod allergens have been identified by the Allergen Nomenclature Sub-committee of the World Health Organization (WHO) [68]. Edible insects may also cause cross-reactivity

in people allergic to seafood. The following allergens are most frequently identified in edible insects: fructose-bisphosphate aldolase, phospholipase A, hyaluronidase, arginine kinase, myosin light chain, tropomyosin, α -tubulin, and β -tubulin [69]. A total of 116 allergic reactions to edible insects, mostly grasshoppers, locusts, and lentil weevils, have been identified in 2018 [68]. Insect allergens induce non-specific symptoms, such as anaphylaxis, allergic asthma, hypotension, gastrointestinal symptoms, loss of consciousness, urticaria, erythema, pruritus, and tachycardia. Employees of insect farms and insect processing plants can also develop allergic reactions [70][71]. Allergies also pose a threat to companion animals. Insects can also harbor foreign allergens [69][72], including mites and their metabolites. Direct contact with new proteins or symbiotic organisms can trigger heightened immune response. Presence of gluten in digestive tracts of insects fed grain [73] can pose a threat to people who suffer from celiac disease. Allergizing potential of edible insects should be monitored to eliminate these risks. Potential allergens in insect-based foods should be clearly listed on the product label.

Prions pose a significant biological hazard. Prions are one of the key hazards that have been identified by the European Food Safety Authority (EFSA) in the risk profile of edible insects [34][43]. Insect-specific prion diseases have not been identified because insects lack the gene encoding the prion protein PrP [34][74]. However, insects may act as vectors for prions from contaminated substrates derived from ruminants, which could pose a risk for humans, companion animals, and livestock [34][43].

At present, there is no scientific evidence to suggest that insects pose a viral risk to consumers [43][44][75][76]. Entomoviruses are not pathogenic to humans. Insects are commonly infected with viruses of the family Baculoviridae, which are not dangerous for humans or animals [36][77]. Humans do not harbor insect-specific viruses, and there is negligible risk that new mammalian-specific virus strains will evolve through recombination and reassortment and lead to host switching, as was the case with Swine flu [36]. Edible insects are unlikely to transmit foodborne viruses, such as Hepadnaviridae (hepatitis A and E), Reoviridae (reoviruses), and Caliciviridae (noroviruses) [50]. However, viruses could be transmitted to insects through feed or through contact with farm personnel. Viruses of the family Rhabdoviridae, which cause vesicular stomatitis, have been reported in edible insects [54]. Risk of SARS-CoV-2 transmission by edible insects is very low [36][76][78]. According to Doi et al. [36], risk of infection with SARS-CoV-2 as a foodborne pathogen is negligent in people who consume edible insects [36]. It should be noted that viruses causing foodborne diseases do not replicate in arthropods [44][75], but edible insects could become contaminated during processing and distribution.

Bacteria are presently regarded as the greatest safety hazard in production of edible insects [47]. Due to physiological, environmental, and behavioral differences, every species of edible insects intended for food and feed production harbors different bacteria [66][79]. According to the literature, the microbiome of edible insects poses a negligent risk to consumer safety [80][81]. Several bacteria that can act as opportunist pathogens in humans have been identified in edible insects, but these pathogens are specific to mammals [66]. The risks associated with bacterial symbionts in insects or their potential effects on vertebrates have not been evaluated to date. Insects can act as vectors and carriers of microorganisms that are harmful to humans, particularly when biosecurity and hygiene standards are not observed in insect farms. Insects can carry bacteria that are dangerous to humans, companion animals, and livestock and can act as vectors of foodborne pathogens [82]. Insect microbiota typically include the following bacterial families and genera: Enterobacteriaceae (*Proteus* spp., *Escherichia* spp.), *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Micrococcus* spp., *Lactobacillus* spp., and *Acinetobacter* spp. [83]. Some species of the above families and genera are potentially pathogenic to humans, whereas others are commonly encountered in healthy subjects. Unprocessed insects and insect-based foods can harbor *Campylobacter* spp., verotoxic *E. coli*, *Salmonella* spp., and *L. monocytogenes* if microbial inactivation techniques are not applied in production plants. Therefore, insects and insect-based foods should always be screened for these pathogens. Prevalence of some of these pathogens is lower in insects than in other animal protein sources. For example, *Campylobacter* spp. is not replicated in the digestive tract of insects [84][85][86]. Similar risks can be encountered during insect processing. Several bacterial species identified in edible insects can shorten the shelf-life of the final product. Presence of spore-forming bacteria in the end product poses one of the greatest bacteriological hazards [87]. Common sanitation practices, such as drying, boiling, or deep frying, may not be sufficient to eliminate these pathogens.

Entomopathogenic fungi are yet another group of potentially hazardous organisms. There is no scientific evidence to suggest that entomopathogenic fungi pose a risk to vertebrates. In some cultures, these fungi (such as *Ophiocordyceps sinensis*) have long been used in traditional medicine [41]. Mycosporidia could also pose a health threat to consumers [88], but their toxicity has not been analyzed to date. According to the literature, microsporidia *Trachipleistophora* spp. that probably originated from insects can infect vertebrates [89][90]. Due to specific insect rearing conditions and administered feeds, the end product can become contaminated with mycotoxins [91][92]. High concentrations of mycotoxins, such as deoxynivalenol, can lead to gastrointestinal dysfunction in mammals. Molds can also develop in insect-based products that have been stored and distributed in sub-optimal conditions. However, presence of molds in insect-based products has

not been reported in the literature. Risks associated with fungi and mycotoxins in insect-derived foods are often disregarded, and further research is needed to guarantee safety of the end product.

Edible insects can potentially transmit parasitic diseases [38][93]. It appears that entomopathogenic parasites are unable to complete their full life cycle in humans or livestock due to biological specificity of the host. Entomopathogenic parasites cannot be transmitted between vertebrates either. However, there is evidence to suggest that some insect-specific parasites can cause digestive problems (such as horsehair worms, *Gordius* spp.) [94] or allergies (*Lophomonas blattarum*) [95]. Insects can also act as intermediate hosts for foodborne pathogens, including tapeworms (*Hymenolepis* spp.), lancet liver flukes (*Dicrocoelium dendriticum*), and nematodes (*Spirocerca lupi*) [93][96][97][98]. Insects can also act as mechanical vectors for different developmental stages of vertebrate parasites in different stages of their life cycle [38][99]. Insects can transmit parasites that colonize body surfaces (hairs, chitin exoskeletons) and digestive tracts. Mechanical transmission of parasites is a serious concern during insect farming. Research has demonstrated that insects can transmit protozoa [93][100][101]. It should also be noted that insects themselves can act as etiological factors of disease. Beetles of the family Tenebrionidae, such as yellow mealworms (*T. molitor*) and lesser mealworms (*A. diaperinus*), can cause canthariasis [102][103][104]. Insect farms can also be colonized by mites [105]. **Table 2** provides a summary of potential biological hazards.

Table 2. Biological hazards associated with different species of edible insects.

Type of Hazard	Infectious Agent	Sensitive Species	Predisposing Factors	References
Prion vectors	Proteinaceous infectious particles	All species fed contaminated substrates of animal origin	<ul style="list-style-type: none">• inadequate rearing practices• failure to observe legal regulations• contaminated feed and litter• handling operations• absence of biosecurity measures• sanitation requirements are not observed by farm personnel• insects are reared with other animals	[34][74]
Viruses	Caliciviridae Hepadnaviridae Vesicular stomatitis virus (VSV)	Migratory locust (<i>Lo. migratoria</i>), black soldier fly (<i>H. illucens</i>) Insects harvested from the natural environment	<ul style="list-style-type: none">• absence of biosecurity measures• sanitation requirements are not observed	[50][54]

Type of Hazard	Infectious Agent	Sensitive Species	Predisposing Factors	References
Bacteria	<i>Aeromonas hydrophila</i> , <i>B. cereus</i> , <i>Clostridium difficile</i> , <i>Clostridium perfringens</i> , <i>Clostridium septicum</i> , <i>Clostridium sporogenes</i> , <i>E. coli</i> , <i>Enterococcus faecium</i> , <i>Enterococcus faecalis</i> , <i>Listeria</i> spp., <i>Salmonella</i> spp., <i>S. aureus</i> .	Migratory locust (<i>Lo. migratoria</i>) Yellow mealworm (<i>T. molitor</i>) Lesser mealworm (<i>A. diaperinus</i>) House cricket (<i>Ach. domesticus</i>) Domestic silk moth (<i>B. mori</i>) Insects harvested from the natural environment	<ul style="list-style-type: none"> handling operations deviations from production standards rearing conditions inadequate rearing practices contamination of feed and litter 	[64][65][66][67][83]
Fungi and mycotoxins	<i>Aspergillus fumigatus</i> , <i>Aspergillus sclerotiorum</i> , <i>Cladosporium</i> spp., <i>Penicillium</i> spp., <i>Fusarium</i> spp., <i>Phycomycetes</i> spp., Microsporidia	Migratory locust (<i>Lo. migratoria</i>) Black soldier fly (<i>H. illucens</i>) Yellow mealworm (<i>T. molitor</i>)	<ul style="list-style-type: none"> high humidity contamination of feed and litter high water activity in the end product inadequate storage conditions insects as vectors of parasitic infections insects as intermediate hosts insects harvested in the natural environment 	[28][48][91][106]
Parasites	Protozoa (<i>Balantidium</i> spp., <i>Cryptosporidium</i> spp., <i>Entamoeba</i> spp.) Trematoda (<i>Dicrocoelium</i> spp., <i>Lecithodendriidae</i>) Cestoda (<i>Hymenolepis</i> spp., <i>Raillietina</i> spp.) Nematoda (<i>Gordius</i> spp., <i>Spirocerca</i> spp.)	Yellow mealworm (<i>T. molitor</i>) Lesser mealworm (<i>A. diaperinus</i>) House cricket (<i>Ach domesticus</i>) Insects harvested from the natural environment	<ul style="list-style-type: none"> absence of biosecurity measures dirty and contaminated feed (such as unwashed vegetables) presence of pests farm/processing personnel do not observe sanitation requirements insects are reared with other animals 	[4][93][94][95][96][97][98][99][100][101]

Type of Hazard	Infectious Agent	Sensitive Species	Predisposing Factors	References
Mites	<i>Acarus</i> spp., <i>Dermatophagoides</i> spp., <i>Goheria</i> spp. <i>Tyrophagus</i> spp.	Mealworm (<i>T. molitor</i>) Lesser mealworm (<i>A. diaperinus</i>) Black soldier fly (<i>H. illucens</i>) House cricket (<i>Ach. domesticus</i>)	<ul style="list-style-type: none"> • feed substrates are contaminated with mites in different stages of the life cycle • biosecurity measures are not observed • sanitation requirements are not observed • high humidity • residual feed is not removed from farm premises 	[105]

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