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Novel foods: a risk profile for the house cricket (*Acheta domesticus*)

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Abstract

Novel foods could represent a sustainable alternative to traditional farming and conventional foodstuffs. Starting in 2018, Regulation (EU) 2283/2015 entered into force, laying down provisions for the approval of novel foods in Europe, including insects. This Approved Regulation establishes the requirements that enable Food Business Operators to bring new foods into the EU market, while ensuring high levels of food safety for European consumers. The present risk profile tackles the hazards for one of the most promising novel food insects, the house cricket (*Acheta domesticus*). The risk profile envisages a closed *A. domesticus* crickets rearing system, under Hazard Analysis and Critical Control Points (HACCP) and good farming practices (GFP), in contrast with open cricket farms. The methodology used involves screening the literature and identifying possible hazards, followed by adding relevant inclusion criteria for the evidence obtained. These criteria include animal health and food safety aspects, for the entire lifespan of crickets, based on the farm to fork One Health principle. When data were scarce, comparative evidence from close relatives of the *Orthoptera* genus was used (e.g. grasshoppers, locusts and other cricket species). Nevertheless, significant data gaps in animal health and food safety are present. Even if HACCP-type systems are implemented, the risk profile identifies the following considerable concerns: (1) high total aerobic bacterial counts; (2) survival of spore-forming bacteria following thermal processing; (3) allergenicity of insects and insect-derived products; and (4) the bioaccumulation of heavy metals (e.g. cadmium). Other hazards like parasites, fungi, viruses, prions, antimicrobial resistance and toxins are ranked as low risk. For some hazards, a need for additional evidence is highlighted.

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1. Introduction

Insects represent a significant part of the diet for many communities and are consumed in several countries in Africa, South America, Asia and Oceania. However, in western markets, the consumption of insects (entomophagy) is yet to be culturally and socially accepted (House, 2016). Insect biodiversity is huge, with estimations ranging from 2.6 to 7.8 million species (Stork et al., 2015). This biodiversity implies a huge diversity of metabolic pathways and microbiomes among the different species. Currently, more than 2,111 documented arthropods are being eaten worldwide (Jongema, 2017). Most consumed arthropods, ordered according to their consumption, belong to these eight groups: Coleoptera (beetles, often the larvae) (31%), Lepidoptera (caterpillars) (17%), Hymenoptera (wasps, bees and ants), Orthoptera (crickets, grasshoppers and locusts) (14%), Hemiptera (true bugs) (11%), Isoptera (termites) (3%), Odonata (dragonflies), Diptera (flies) and others (9%) (van Huis, 2018). However, only limited numbers of insect species are reared on a large scale as food and feed. According to some market studies, Europe is becoming the fastest growing market for edible insects, forecasting revenues of US$1.07 billion in 2022 (Persistence Market Research, 2018). The same sources highlight that the order Orthoptera is expected to advance even faster, due to the high demand for cricket-based products (e.g. protein powder, granola bars, crackers or cookies) (TECA, 2013).

From a nutritional point of view, insects have an interesting nutritional profile, offering important sources of vitamins, minerals and animal-derived proteins (Wang et al., 2004). They also require less feed for each kg of food produced, and have higher relative growth and lower emission of greenhouse gases (GHG) compared with pigs and cattle (Oonincx et al., 2010; Oonincx and de Boer, 2012). *Acheta domesticus* requires 1.7 kg of dried feed to produce 1 kg of food, compared with 2, 3.8 and 7 kg for poultry, pigs and cattle, respectively (Paoletti, 2005). According to the Food and Agricultural Organization (FAO) predictions, an increase of 70% of the global agricultural production will be needed to fulfil the expected demand. Considering their efficiency, edible insects could play an important role to meet this increasing demand, in particular as an important source of animal protein (FAO – High Level Expert Forum, 2009).

In some countries, insect consumption stands as common practice with a well stabilised industry. As an example, Thailand has released the first Good Agricultural Practices (GAPs) for cricket rearing (ACFS, 2017). According to European regulations and guidelines, insects should be considered as livestock. Hence, good farming practices (GFP) already enforced for other animal husbandry, such as swine, cattle or poultry should be applied. Despite this scenario, and due the particularities of insect rearing, those farming practices should be revised and adapted.

With Novel Foods Regulation (EU) 2283/2015 that entered into force in January 2018, insects and insect-derived products are considered to be novel foods and are subject to the novel foods approval procedure. General health risks associated with consumption of insects have already been tackled in several published risk profiles and scientific opinions (FAO, 2013; EFSA Scientific Committee, 2015; Finke et al., 2015; Schafer et al., 2016). However, due the huge diversity within the insects' world, there is a need to target insect species relevant for European consumers specifically. Therefore, a specific risk profile for the consumption of *A. domesticus* reared in controlled conditions has been developed (Fernandez-Cassi et al., submitted).

2. Description of the work programme

2.1. Aims

The aim of this work is to present a specific risk profile for the house cricket (*A. domesticus*) intended for human consumption. The risk profile will present the current knowledge available on house cricket as food. Also, during the development of the work, several data gaps have been identified. Risks will be ranked as low, medium or high according to information available in the scientific literature considering, in a qualitative manner, the probability that a genuine hazard exists and the consequences of exposure. A hazard is ranked as 'low' when measures can be applied during processing and before consumption, to decrease or inactivate/destroy the hazard. In a similar manner, a hazard is ranked 'medium' when measures applied are insufficient to guarantee the complete removal of the hazard or important data gaps about the likelihood or the consequences of being exposed to it exist. Finally, a hazard is ranked 'high' when its exposure can have serious consequences or it is very likely to happen despite measures applied during processing (EFSA BIOHAZ Panel, 2012).

2.2. Methodology

The literature was scanned and retrieved through searches using the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed), Google Scholar (https://scholar.google.com), Scopus® (https://www.scopus.com) and Web of Science (https://apps.webofknowledge.com). Selection of the included papers was carried out stepwise. Initially, the article titles from the literature were assessed for inclusion prior to reading the abstracts. So, if the abstracts were deemed relevant, the full paper was retrieved and read. Websites belonging to relevant organisations and authorities (e.g. World Health Organization, European Food Safety Authority) were also used to retrieve information. The literature search was carried out between October 2017 and April 2018. The search focused on available scientific evidence for insects as food. Field studies using cricket species as animal models were also included as scientific evidence. Due to the reduced volume of published articles on *A. domesticus* as food intended for human consumption, efforts have been made to identify similarities with crickets used as pet food, different cricket species and including other insects in the order Orthoptera.

3. Biological hazards

3.1. Bacteria and antimicrobial resistance genes

Currently, no specific microbial criteria are available in the European legislation for whole insects or insect-based products intended for human consumption. Some authors (e.g. Caparros Megido et al., 2017) have suggested using total aerobic counts (TAC) of minced meat as food safety and final product hygiene guidelines values (according to European Commission (EC) Regulation No. 2073/2005). However, the numbers provided for minced meat (5×10^5 CFU/g) are difficult to match in non-treated crickets according to published microbial loads (Table 1). The fact that whole animals are eaten, including their guts, which can contain around 10^6–10^{12} bacteria per mL might explain the high reported values (Cazemier et al., 1997). To decrease the high microbial counts, some farmers apply 24–48 h fasting prior to the killing step. However, the efficiency of this procedure to decrease microbial load is uncertain. Literature TAC values range from 10^4 CFU/g to 10^8 CFU/g. Different protocols used or the application of processing treatments such as heat-treated crickets might explain the different numbers reported.

Despite high microbial loads, food-borne bacteria such as *Listeria monocytogenes* have never been reported. Other important species, such as *Salmonella* spp. or *Escherichia coli*, have been rarely reported by plating (Caparros Megido et al., 2017; Grabowski and Klein, 2017a; Osimani et al., 2017; Vandeweyer et al., 2017a). Despite not being natural reservoirs for well known food-borne bacteria, crickets might be contaminated during processing (i.e. during farming, packaging, cooking or serving). *Yersinia* spp., *Citrobacter* spp., *Fusobacterium* spp. and *Bacteroides* spp. have been documented in previous studies in crickets (Ulrich et al., 1981).

Crickets are a suitable environment for bacterial regrowth according to reported pH and water activity (a_w) values (Vandeweyer et al., 2017a, 2018). Therefore, light heat treatments, such as blanching (i.e. for 1 min), might reduce microbial counts, but does not prevent rapid spoilage when insect products are stored at room temperature, as the environmental conditions favour bacterial regrowth taking advantage of the high-water content, favourable pH and nutrient-rich environment (Klunder et al., 2012). Intensive blanching treatments (4 min) combined with a rapid cooling procedure appear to ensure compliance with TAC levels for minced meat (Klunder et al., 2012). Despite the obtained numbers, the authors recommend boiling for 10 min to ensure acceptable microbial loads. Grabowski and Klein (2017c) assessed microbial loads in differently thermally processed *A. domesticus* products. Intense heat-treated products (deep-fried, dried and extruded) were compliant with the thresholds for TAC and Enterobacteriaceae in minced meat suggested by some competent authorities. However, powdered and dried insect products would require additional thermal processing before consumption to comply with the same TAC values.

Temperature/time combinations provided up to now might be insufficient to destroy sporulated bacteria (ANSES, 2015). In crickets, sporulated bacteria are described in the range of 10^2–10^5 CFU/g, depending on the study and the product analysed (Osimani et al., 2017; Vandeweyer et al., 2017a). Similarly, other sporulated bacteria, such as *Bacillus cereus*, were detected in grasshoppers in 88% of samples tested (15 out of 17), in counts lower than 10^2 CFU/g (NVWA, 2014). *B. cereus* has also been identified in *A. domesticus* extruded products (Grabowski and Klein, 2017c). *Clostridium perfringens* and other sulfite-reducing clostridia have rarely been detected, or were found in low concentrations (10^2 CFU/g) (Osimani et al., 2017). The removal of indigenous microbiota, for example by short

blanching treatment, could render the food vulnerable to spore-forming bacteria, leaving them free to grow without competition. Some of the mentioned species such as *Clostridium* spp. and *Bacillus* spp. could produce thermally stable toxins. On the storage of crickets' products, Vandeweyer et al. (2018) observed that microbial loads remained stable in different processed *Gryllodes sigillatus* products on a 6-month survey.

The advent of DNA sequencing technologies, such as high-throughput sequencing (HTS), have allowed the study of microbial communities of reared insects. For example, Garofalo et al. (2017) identified a low abundance of reads, taxonomically assigned to *Clostridium* spp., *Staphylococcus* spp., *Listeria* spp. and *Bacillus* spp., previously not detected by culturable methods. High-throughput methodologies rely on the DNA present in a given sample. Therefore, non-viable or viable non-culturable bacteria (VBNC) can still be detected. The analysis of the 16S subunit of ribosomal RNA is an useful tool for taxonomic assignment up to the genus level, but lacks sensitivity to reach the species level (Poretsky et al., 2014). Vandeweyer et al. (2017b) used a similar approach by studying reared crickets from three different establishments, while assessing microbial loads by culturable methods. Interestingly, the different batches reared in the same facilities presented different bacterial loads. In general, it seems that crickets present a high microbial diversity with a high relative abundance of minority operational taxonomic units (OTUs) (Vandeweyer et al., 2017b, 2018). The different rearing companies presented different OTUs profiles, suggesting that microbial communities are dependent on rearing conditions and are highly influenced by dietary and environmental factors (e.g. manipulation by breeders, food and water microbiota).

Insects can act as vectors for antimicrobial resistance genes (AMR). Milanović et al. (2016) studied the presence of AMR genes by using polymerase chain reaction (PCR) or nested-polymerase chain reaction (n-PCR) in edible insects. Tetracycline resistance genes (*tet* (K), *tet* (M) and *tet* (O)) were detected in cricket samples. The study showed different principal coordinates analyses (PCA) for AMR profiles of the insects reared in Europe compared with ones reared in Thailand. These results might reflect the different selective pressure caused by sanitisers used on different rearing companies on microorganisms carried by edible insects. Finally, these results suggest the possibility of using insects as sentinels for AMR in the environment.

3.2. Fungi, mycotoxins, yeasts and moulds

Insects are affected by most species of fungi and their presence is subject to several influencing factors (Boomsma et al., 2014). Visible fungi have been documented by breeders in insect-farming facilities (FAO, 2013). The presence of visible fungi has been also reported in breeding experiments at the Swedish University of Agricultural Sciences (SLU) without involving any major mortality or incidence.

Caparros Megido et al. (2017) found that yeast and mould counts for crickets were above the Good Manufacturing Practice (GMP) limits for raw meat. However, the addition of a heat treatment such as blanching reduced the yeast and mould counts to acceptable GMP levels. Comparatively, reared *G. sigillatus* crickets presented fungi isolates from the genera *Aspergillus*, *Candida*, *Kodamaea*, *Lichtheimia*, *Tetrapisispora*, *Trichoderma* and *Trichosporon* (Vandeweyer et al., 2018). The use of the denaturing gel gradient electrophoresis (DGGE) technique for cricket powder and small crickets allowed the detection of several fungi from the genera *Aspergillus*, *Tetrapisispora*, *Eurotium* and *Wallemia*. Yeasts from the genus *Debaryomyces* were detected in the same study. Most of the reported fungi genera are commonly found in soil and water (Guarro, 2012), but some are also involved in sporadic invasive or superficial infections (Roussel et al., 2004; Hubka et al., 2012).

Some fungi, such as *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp., can produce mycotoxins that have serious consequences for human health (Bennett and Klich, 2003). Vandeweyer et al. (2018) isolated mycotoxin-forming fungi from *Aspergillus* spp. and *Penicillium* spp. from the feed, substrate and/or within *G. sigillatus*. Noteworthy, once mycotoxins are present they are difficult to remove as some of these are thermally stable (Magan and Olsen, 2004). Other fungi species of the genus *Eurotium*, reported by Grabowski and Klein, produce echinulin and neoechinulin, which have been suggested to be toxic for animals (Ali et al., 1989; Pitt and Hocking, 2009). The potential toxicity of mycotoxins for insects is uncertain. Surprisingly, some aphid species have been documented to transform and detoxify mycotoxins produced by *Fusarium* (i.e. trichothecene deoxynivalenol (DON); De Zutter et al., 2016). In a similar way, other insect species might have biochemical pathways to detoxify mycotoxins (Camenzuli et al., 2018). More research is needed to assess the presence of mycotoxin-producing fungi in edible insects and their possible detoxification by insects including *A. domesticus*.

3.3.   Parasites

Considering the present knowledge, no human parasites found in reared crickets have been described in the literature. Recently, some scientists have hypothesised about the possibility that *Abbreviata antarctica*, a lizard parasite that might have crickets as intermediate host, could infect human causing under-reported cases due to lack of knowledge (King and Jones, 2016).

Insects might transfer parasitic cysts from faeces to foods, so acting as vectors. However, all infective stages of parasites are destroyed by suitable heat treatments (Doyle, 2003). For example, food control of *Toxoplasma gondii*, a well-known zoonotic parasite, involves cooking meat at a temperature of at least $+66°C$ (Dubey et al., 1990), or freezing it at $−12°C$ (Dubey, 1996). Although an unexplored field that would merit further research, it seems reasonable to classify parasites as a low risk hazard.

3.4.   Viruses

Insects are susceptible to be infected by a huge diversity of virus species. However, limited data are available about insects' virome. Shi et al. (2016) explored the transcriptome of more than 220 invertebrates, including crickets. Based on the analysis of the RNA-dependent RNA polymerase (RdRp), they discovered 1,445 different RNA viruses, some of these were sufficiently divergent to be considered as new families. Some virus families that infect insects are shared with humans and are well known human pathogens (*Poxviridae*, *Parvoviridae*, *Picornaviridae*, *Orthomyxoviridae* and *Reoviridae*) (EFSA, 2015).

Virus infections are a main concern for insect farmers, as they might induce high mortality rates, leading to economic losses. The cricket paralysis virus (CrPV) of the *Dicistroviridae* family, and the cricket densovirus (AdDV) from the *Parvoviridae* family are considered two of the most important virus pathogens for crickets (Maciel-Vergara and Ros, 2017). These virus families contain human pathogens, raising the concern of their pathogenicity for humans if the viruses cross the species barrier. The scientific evidence for the inability of insect viruses to infect vertebrate cell lines in combination with the high evolutionary distance between host taxa suggest an unlikely threat for human health (El-Far et al., 2004). No major human food-borne viruses, such as noroviruses and hepatitis A and E viruses, have been reported in insects. Considering the long phylogenetic distance between humans and crickets, their replication in crickets seems unlikely. The lack of hygienic measures during insect rearing (e.g. soil, water or feed contaminated with faeces) could represent an entrance point of human virus particles into the food chain. Also, the possibility that insect-based products could be contaminated during processing or handling could not be discarded. The survival of food-borne viruses via the gut of the crickets represents an important data gap and should be addressed by future studies. Although out of the scope of the present risk profile, we cannot exclude the scenario of crickets acting as mechanical vectors, when exposed to contaminated environment or feed. Finally, no single arbovirus that could constitute a human health threat has been detected in crickets.

3.5.   Prions

Prions have been one of the main concerns in animal health and food safety over the last decades. Prion codifying genes or gene orthologues have not been detected in insects, making crickets naturally prion free (Thackray et al., 2012). This situation implies that the amplification/replication of prion proteins is impossible within crickets. However, their role as mechanical vectors should not be discarded (Post et al., 1999). Prions are highly stable in the environment, persisting as infective for long periods in water and soil (Maluquer de Motes et al., 2008; Smith et al., 2011). This high stability suggests the possibility of remaining infective for humans if previously ingested by insects. Hence, it is important to control the quality of feed used for cricket rearing, as well as complying with the feed provisions laid down in Commission Regulation (EU) 1148/2014, amending Regulation (EU) 999/2001, to avoid the entrance of prions into the cricket food chain. Recently, the legislation has been amended, relaxing the feed bans on the use of insect processed animal proteins (PAPs) for aquaculture animals, via Regulation (EU) 893/2017. Taking the available data into consideration, we can conclude that prions do not represent an important cause of concern for the envisaged food system.

4.   Chemical hazards

4.1.   Heavy metals

Crickets, as other food products, may contain cadmium, arsenic, lead and tin, but few studies have evaluated their presence. The concentration of heavy metals in crickets is dependent on their presence in animal feed or soil pollutants. Heavy metals can be bioaccumulated or bioconjugated. According to Bednarska et al. (2015), crickets are more efficient in regulating their dietary exposure to zinc than to cadmium, suggesting that crickets tend to accumulate cadmium. This hypothesis is supported by other authors, using data from other species from the *Orthoptera* genus (Devkota and Schmidt, 2000; Vijver et al., 2003; Zhang et al., 2009). Studies analysing the concentration of mercury and its organic forms in insects or crickets intended for food consumption are rare. Insects have, however, been proposed as sentinels to monitor the level of contaminants in the environment (Ortiz et al., 2015). Using these studies, it has been suggested that mercury concentration in crickets is influenced by their dietary/ environmental exposure (Zhang et al., 2009; Rimmer et al., 2010). According to reported data, it appears that, under a controlled rearing process, low risk exists for mercury bioaccumulation. For other metals, such as lead, a low bioaccumulation for grasshoppers was reported in comparison with mercury or cadmium (Devkota and Schmidt, 2000). Moreover, this study also suggested that cadmium was more easily absorbed due to its higher chemical activity compared to lead.

The concentrations of heavy metals in edible insects or insect-derived products have been explored by Poma et al. (2017), including cricket-derived products. Concentrations of all tested heavy metals (cadmium, arsenic, chromium, lead and tin) were within the acceptable levels for human consumption.

Data from heavy-metal bioaccumulation in other insect species are available. However, the extrapolation of these data to crickets could be inaccurate, as important different metabolic and physiological differences exist between insect species. Seasonal variations in metal concentrations, as well as differences due to developmental stages, might play a role in the bioaccumulation phenomenon (Janssen et al., 1993). The presence of heavy metals, such as arsenic, aluminium, cadmium, chromium and mercury, in edible insects used for human consumption merits further research. Based on the few available studies, the levels detected in insect foodstuffs are compliant with Regulation (EU) 1881/2006 for contaminants.

4.2.   Toxins and antinutrients

Insects can contain naturally toxigenic compounds or antinutrients for humans. Toxigenic compounds can be synthesised as defensive mechanisms or accumulated during the rearing processes. No internal toxins for humans are described in crickets (EFSA, 2015). Koc et al. (2014) performed a genotoxicity study using the water-soluble extracts of commercially available mole crickets, *Gryllotalpa* spp. were cultured human blood cells, and used for the micronucleus test to monitor DNA and chromosomal damage. The study concluded that cricket extracts have no genotoxic effects at tested concentrations (0–2,000 ppm). Similarly, crickets do not have specific organs to produce toxic compounds (phanerotoxic), nor can they bioaccumulate toxins (cryptotoxic) (EFSA, 2015; van der Spiegel et al., 2013). No case of acute intoxication due to toxins in crickets has been reported (FASFC, 2014). Rat-based animal studies using cricket powder determined a no-observed-adverse-effect-level (NOAEL) dose over 5,000 mg/kg without any adverse effect in a 13-week oral toxicity study (Ryu et al., 2016). The results point out that crickets could be suitable as food from a toxicological point of view. Currently, there is no single antinutritive compound identified in crickets. It is possible that fractioned products of crickets could be enriched in antinutrients or toxicological compounds that might have been unnoticed and represent a health problem in the future. Data on the toxicity of edible crickets and insects are scarce, representing a data gap that should be explored.

4.3.   Dioxins, organochloride compounds, flame retaining compounds, polycyclic aromatic hydrocarbons and other chemical compounds

The presence of dioxins (polychlorinated biphenyls (PCBs)) and dioxin-like (DL-PCBs) in insects is an unexplored field. Paine et al. (1993) studied the concentration of PCBs in reared crickets without direct contact with soil in a naturally PCB polluted environment. Results suggest that PCBs are quickly absorbed by crickets but not accumulated. Other studies suggest that the *Orthoptera* genus is less efficient in bioaccumulation of PCBs compared with *Coleoptera* (Blankenship et al., 2005). Poma et al.

(2017) tested insects and insect-derived products placed on the market for 12 different PCBs compounds. The concentration detected in cricket-derived products showed that they were within the safe margins of PCBs levels according to EU legislations. In the same study, the insecticide organophosphorus pirimiphos-methyl was detected. Its presence could be attributed to the composition of cricket-derived products tested that had a high vegetable content. Finally, the possibility that other chemical compounds [e.g. heterocyclic aromatic amines (HAAs), polycyclic aromatic hydrocarbons (PAHs), chloropropanols, furans or acrylamide] could be generated due to chemical reactions between insect compounds and other ingredients during processing should not be discarded. This possibility will merit further studies and represents a gap in the scientific data (van der Spiegel et al., 2013).

4.4. Allergies

According to the World Health Organisation and the International Union of Immunological Societies (www.allergen.org, last accessed 19/01/2018), there is no single allergen reported under the order *Orthoptera* (crickets). Specific food-borne allergies derived from cricket consumption have not been notified in Europe. Likewise, allergic reactions linked to *A. domesticus* are rarely reported in regions where cricket consumption is more common. Cross-reactivity allergic reactions among crickets with other arthropods has been suggested (Panzani and Ariano, 2001). Cross-reactivity is based on the existence of commonly conserved (glycol) proteins present in different species (pan-allergens). With an increasing consumption of insects, an increase in allergic reactions against arthropods is predicted (i.e. shrimp, crab) as crickets share high protein homologies with arthropods. For example, tropomyosin, which is a well-known allergen in crustacean, is also present in crickets. So, people who are allergic to crustaceans can be sensitive to crickets and, with repeated exposures, prone to develop an allergic reaction. In those sensitised individuals, the consumption of crickets could therefore trigger allergic reactions as if they were exposed to the original allergen animal (e.g. shrimps). There is documented cross-reactivity with other arthropods (such as shellfish), with world-wide estimated prevalence as high as 10% (Moonesinghe et al., 2016). Therefore, cricket and cricket-derived products should be labelled to ensure safety of consumers allergic to crustaceans or molluscs (FASFC, 2014). Similarly, other important pan-allergens such as arginine-kinase (AK) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are present in crustaceans (i.e. shrimp) and insects (Chuang et al., 2010; Khanaruksombat et al., 2014). Hexamerin B1 has been identified as an specific allergen of *Gryllus bimaculatus* (field cricket) (Srinroch et al., 2015).

The presence of fungi from the genera *Aspergillus* and *Penicillium* in insects could trigger secondary allergic reactions (Schlüter et al., 2017). The presence of allergens in insects and insect-based food can be modulated by applied food processing treatments. As an example, heat treatments can alter the protein structure, so triggering or shutting down the allergenicity of specific compounds. This effect has been documented by Phiriyangkul et al. (2015) who described a change in the allergenic profile of a locust species, *Patanga succincta*, when consumed raw or processed (fried). Similarly, the allergenic profile of crickets could be substantially different depending on the food processing technique used.

5. Conclusions

According to available scientific data, viruses, prions, fungi and parasites should be considered as low risk hazards. High microbial load, spore-forming bacteria and its regrowth after heat treatment, heavy metals bioaccumulation (in special cadmium) and allergenicity of crickets are considered the medium hazards. More research is needed to evaluate the safety of crickets as food intended for human consumption to cover identified data gaps (e.g. mycotoxins or chemical compounds such as heavy metals or dioxins on edible crickets placed on the market).

Crickets as food show higher microbial loads compared with other food products. Therefore, specific hygiene and safety criteria values for insects, including crickets, should be developed.

Commonly detected food-borne pathogenic bacteria, such as *L. monocytogenes or Salmonella* spp., have never been or are rarely reported in crickets intended for human consumption. Still, the use of HTS technologies has allowed the description of the cricket's microbiota and detected sequences taxonomically classified to the genera *Clostridium* spp., *Listeria* spp. and *Bacillus* spp., which contain relevant food-borne pathogens.

Thermal treatments, such as blanching, boiling or frying, can decrease microbial loads in edible insects. A mandatory thermal process for crickets or cricket-derived products should be implemented before product placement on the market. Furthermore, boiling before consumption could be advisable to ensure microbial loads that comply with both hygiene and food safety standards. However, such treatments may not be enough to kill spores from *Bacillus* spp. and *Clostridium* spp.

Heavy metals have been identified as putative chemical hazards when crickets are exposed to these during the rearing stage. Among heavy metals, cadmium bioaccumulation has been identified as a major concern. Available information on other heavy metals, such as aluminium, chromium and arsenic, is scarce and more data are required.

Crickets can trigger allergic reactions in sensitive consumers (e.g. prawns, crabs, lobsters). Homologue proteins shared between different species can trigger pan-allergic reactions. Tropomyosin, AK or GAPDH have been identified as a highly allergenic. Hexamerin B1, whose allergenic potential requires more research, has been described as a specific cricket allergen. For safety reasons, crickets and cricket-derived food products should be labelled to raise awareness in susceptible consumers (Table 2).

Table 1: Microbial loads reported for crickets reared as food, feed and cricket powder. Results are expressed in CFU/g. NT not tested; NEG: negative or under the limit of detection for the technique and microorganism

Product	Total aerobic counts	Enterobacteriaceae	Aerobic bacterial endospores	Moulds	Yeast	Reference
Whole crickets	3.16×10^8	1.6×10^7	5.01×10^3	4.0×10^5	4.0×10^5	Vandeweyer et al. (2018)[a]
Whole crickets	2.1×10^4	NEG	NT	NEG	7.9×10^4	Garofalo et al. (2017)[c]
Cricket powder	3.6×10^4	NEG	NT	1.1×10^3	NEG	
Whole crickets	1.59×10^4	NEG	3.98×10^3	NEG	NEG	Osimani et al. (2017)
Cricket powder	1.00×10^5	1.26×10^3	1.26×10^5	1×10^2	2.00×10^3	
Whole crickets	2.1×10^8	5.5×10^7	6.6×10^3	2.6×10^6	2.6×10^6	Vandeweyer et al. (2017a)[a]
Whole crickets	3.16×10^7	1×10^7	3.16×10^3	NEG	NEG	Grabowski and Klein (2017a,b,c)[a],[b]
Dead whole crickets	5.01×10^7	5.01×10^6	NEG	2.51×10^5	2.51×10^5	
Whole crickets	8.91×10^7	NT	NT	6.31×10^4	6.31×10^4	Caparros Megido et al. (2017)[a]
Cricket powder	8.2×10^4	NT	NT	NT	NT	Milanović et al. (2016)
Whole crickets	1.4×10^4	NT	NT	NT	NT	
Whole crickets	1.59×10^7	1.59×10^4	3.98×10^3	NT	NT	Klunder et al. (2012)

CFU: colony forming unit.
(a): Moulds and yeasts are cultured by using the same assay.
(b): Crickets intended for pet consumption. Crickets were already dead in the rearing facilities.
(c): Insects were crushed but not blended. Insects were boiled, dried and then sold.

Table 2: Microbial loads reported for thermally treated crickets reared as food or feed). Results are expressed in CFU/g. NT not tested; NEG: negative or under the limit of detection for the technique and microorganism

Thermal treatment	Total aerobic counts	*Enterobacteriaceae*	Aerobic bacterial endospores	Moulds	Yeast	Reference
Boiled	3.98×10^2	3.1×10^1	2.51×10^2	NEG	NEG	Vandeweyer et al. (2018)[a]
Frozen	2.51×10^2	NEG	1.0×10^2	NEG	NEG	
Oven dried	1.99×10^4	NEG	2.51×10^2	NEG	NEG	
Smoked and dried	7.94×10^7	NEG	2.51×10^3	NEG	NEG	
Blanching (4 min)	2.46×10^4	NT	NT	NEG	NEG	Caparros Megido et al. (2017)
Sterilised (16 min – 120°C)	5.50×10^3	NT	NT	NEG	NEG	
Freeze dried	1.12×10^4	NT	NT	NEG	NEG	
Boiled (5 min)	5.01×10^1	NEG	3.16×10^1	NT	NT	Klunder et al. (2012)
Stir fried (5 min)	5.01×10^2	NEG	3.16×10^1	NT	NT	

CFU: colony forming unit.
(a): Moulds and yeasts are cultured by using the same assay.

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Abbreviations

AdDV	cricket densovirus
AK	arginine-kinase
AMR	antimicrobial resistance genes

ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (France)
a_w	water activity
cfu	colony forming unit
CrPV	Cricket paralysis virus
DGGE	denaturing gel gradient electrophoresis
DL-PCBs	dioxin-like polychlorinated biphenyls
DON	deoxynivalenol
FASFC	Federal Agency for the Safety of the Food Chain (Belgium)
FAO	Food and Agricultural Organization
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GFP	good farming practices
GHG	greenhouse gases
GMP	good manufacturing practice
HAAs	heterocyclic aromatic amines
HACCP	Hazard Analysis and Critical Control Points
HTS	high-throughput Sequencing
NOAEL	no-observed-adverse-effect-level
n-PCR	nested polymerase chain reaction
NVWA	Nederlandse Voedsel- en Warenautoriteit (Netherlands)
OTUs	operational taxonomic units
PAH	polycyclic aromatic hydrocarbons
PAPs	processed animal proteins
PCA	principal coordinates analysis
PCBs	polychlorinated biphenyls
PCR	polymerase chain reaction
RdRp	RNA-dependent RNA polymerase
RNA	ribonucleic acid
SLU	Swedish University of Agricultural Sciences
TAC	total aerobic counts
VBNC	non-viable or viable non-culturable bacteria