Trends in Parasitology

CellPress REVIEWS

Review

The Challenge of Developing a Single-Dose Treatment for Scabies

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Scabies is a common skin disease with an estimated worldwide incidence of 200 million people infected per year. Its morbidity and mortality is principally due to secondary bacterial infections, a link now well recognized and prompting the recent inclusion of this disease-complex in the WHO list of neglected tropical diseases. The few treatments available are poorly effective against *Sarcoptes scabiei* eggs and appear to induce resistance in the parasite. An ideal alternative would be a single-dose regimen that kills all developmental stages, including eggs. Drugs used in the veterinary field and applied to other arthropods could be tested experimentally in an established pig-scabies model. Moreover, functional genomics combined with target validation through biochemical research should assist in identifying new drugs.

Highlights

Worldwide, approximately 200 million people are infected with scabies each year, and this number appears to be rising.

Scabies is often complicated by infections with opportunistic pathogens, prompting the addition of scabies to the WHO list of neglected tropical diseases (NTDs) in 2018. Although still highly neglected, renewed efforts are now directed toward the global control of scabies.

Recent research indicates that acaricidal drugs used in the veterinary field might be applicable to human scabies.

As current treatments are not highly effective, there is a need for improved chemotherapy, ideally a single-dose oral regimen that kills all developmental stages of *Sarcoptes scabiei*, including the eggs. The use of advanced molecular and biochemical technologies, together with *in vivo* experiments using the pig-scabies model should help to achieve the goal of designing such a new intervention.

Rationale for Developing a New Scabies Treatment Approach

Sarcoptes scabiei, the parasitic mite that causes human scabies, relies on the host epidermis for its nourishment, reproduction, habitat, and survival. There are no free-living development stages and no intermediate hosts in its simple life cycle (Box 1). This offers an ideal opportunity to target all developmental stages with only one treatment. Although in use for decades, current broad-spectrum treatments (Table 1) do not affect the eggs of *S. scabiei*, which means that the life cycle is not completely disrupted, and hatching larvae can rapidly re-establish or exacerbate infestation. Further, the proliferation and desquamation of epidermal cells push eggs away from the basal epidermal layers and thereby away from host defense and exposure to systemic drugs diffusing in from the dermis (e.g., orally administered ivermectin). Unless a non-ovicidal drug has a long enough half-life, or well-timed repeat treatment is applied, the eggs and newly emerged larvae remain unaffected, and can develop further, allowing the infestation to perpetuate.

A next generation of effective **scabicides** (see Glossary) is urgently needed and would, if tailored to simultaneously kill all developmental stages of *S. scabiei*, improve the effectiveness and efficiency of future scabies management strategies and result in reduced scabies recurrence rates. Here we explore acaricide and insecticide molecules that have been recently developed for animals; we review the current knowledge of mite embryology and ovicidal treatments used against other arthropods and give an overview of molecular technologies and an established *in vivo* model, all of which may assist the development of new scabicides.

Current Scabies Treatments for Humans and Their Limitations

Currently, there is no vaccine to prevent scabies [1], and relatively few therapeutic regimes are available for human scabies (Table 1) [2]. No improved or highly effective new treatment regimen has been developed in the last ~30 years [3]. Topical permethrin and systemic/topical ivermectin are conventional broad-spectrum drugs of choice which are still used primarily due to their low cost [4]. Almost all drugs in use kill motile stages (larvae, nymphs, and adult mites) by affecting

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their nerve and muscle functions [5,6], but, as mentioned, do not kill the egg stage (Bernigaud *et al.,* unpublished). We propose that the absence of ovicidal activity becomes a problem if a drug has a short half-life in the skin and the new generation of larvae hatching after treatment survives because the drug concentration has become suboptimal by the time the larvae hatch.

Permethrin (5%) cream is the first-line treatment, used topically, but must be repeated after 1 week to be efficient, which indicates poor or limited ovicidal action, which has been confirmed in recent *in vitro* efficacy experiments. In addition, permethrin has been used as a scabicide in Australia for the past 20 years, and its efficacy appears to be decreasing, possibly due to emerging resistance [7].

Ivermectin is the only available oral drug for scabies treatment. Ivermectin is mainly used in patients with severe, crusted scabies or with superinfected or eczematous skin lesions, in endemic areas or in institutions where outbreaks require the mass administration of an effective compound [8]. The proposal that ivermectin has limited ovicidal activity [9] has been confirmed recently. In a therapeutic trial using oral ivermectin, performed in a porcine model infested with scabies, the half-life of ivermectin in skin was shown to be approximately 1 day after administration of a therapeutic oral dose of ivermectin (of 0.2 mg/kg). After 2 days, the ivermectin concentration in the skin was as low as 60 ng/g, and undetectable after 7 days following administration [10]. A recent *in vitro* study of the susceptibility of *S. scabiei* to ivermectin indicates that these doses are likely insufficient to kill all mites in the skin [11]. Currently, there are no pharmacokinetic data available from human infestations. Given the similar characteristics of porcine and human skin physiology, the findings achieved using the pig-scabies model indicate that a second dose of ivermectin is generally required to kill hatching larvae and to achieve a clearance of *S. scabiei* infestation. Further, a growing concern with the use of ivermectin arises from reports that human scabies populations are displaying resistance to the compound due, in part, to suboptimal treatment efficacy [7,12].

Box 1. Scabies – An Under-Rated Public Health Problem

Human scabies is a contagious infestation of the human epidermis by the microscopic parasitic mite *Sarcoptes scabiei* var. *hominis* (Figure I). Intense **pruritus** is the major symptom resulting in scratching behavior, which disturbs the skin barrier and provides an opportunity for secondary bacterial infections to establish. Superinfection can lead to localized **pyoderma**, abscesses, and cellulitis, or serious systemic illness, such as **bacteremia** and **septicemia**, or postinfective complications including heart and kidney diseases [72,73]. *Streptococcus pyogenes* (group A *Streptococcus*, GAS) and *Staphylococcus aureus* (including methicillin-resistant strains, MRSA) play a major role in exacerbating cutaneous disease caused by the scabies mites [74].

There are two forms of scabies [2]. The first form, classical or common scabies, is found in most patients and is characterized by mild to moderate skin lesions and a mite burden of <20 mites/person, causing a generalized, intense allergic rash [75]. The second, much less prevalent form, is called crusted scabies, which is characterized by hyperkeratotic skin (crusts), typified by 100–1000s of mites per gram of skin [76]. Severe manifestations of scabies are frequently associated with immune suppression [77] that is challenging to treat and potentially life-threatening [78].

The highest prevalence of scabies is seen in young children [79] and in people who are immunocompromised as a result of old age, immunopathological diseases, immunosuppressive drugs, or their genetics [80,81]. Overcrowded housing in endemic regions or in institutions, hospitals, nursing homes, and child- or aged-care centers can provide conditions that are favorable for scabies to spread [82,83]. Disease transmission occurs mainly via close skin-to-skin contact, but transmission via fomites can also occur [84], as *S. scabiei* mites and eggs can survive for several days in the environment, depending on environmental temperature and humidity [68].

Of the 15 most burdensome dermatologic conditions of humans, evaluated in **disability-adjusted life years (DALYs)**, scabies ranks higher than melanoma and psoriasis [85]. Recent reviews [79,86,87] indicate that the actual scabies burden is likely underestimated due to an absence of accurate diagnostic tools and consequent gaps in disease surveillance [88,89]. The recommendation by the WHO to include scabies in the highest neglected tropical disease (NTD) category was realized in 2018, and came with an urgent call for research and drug development [90,91].

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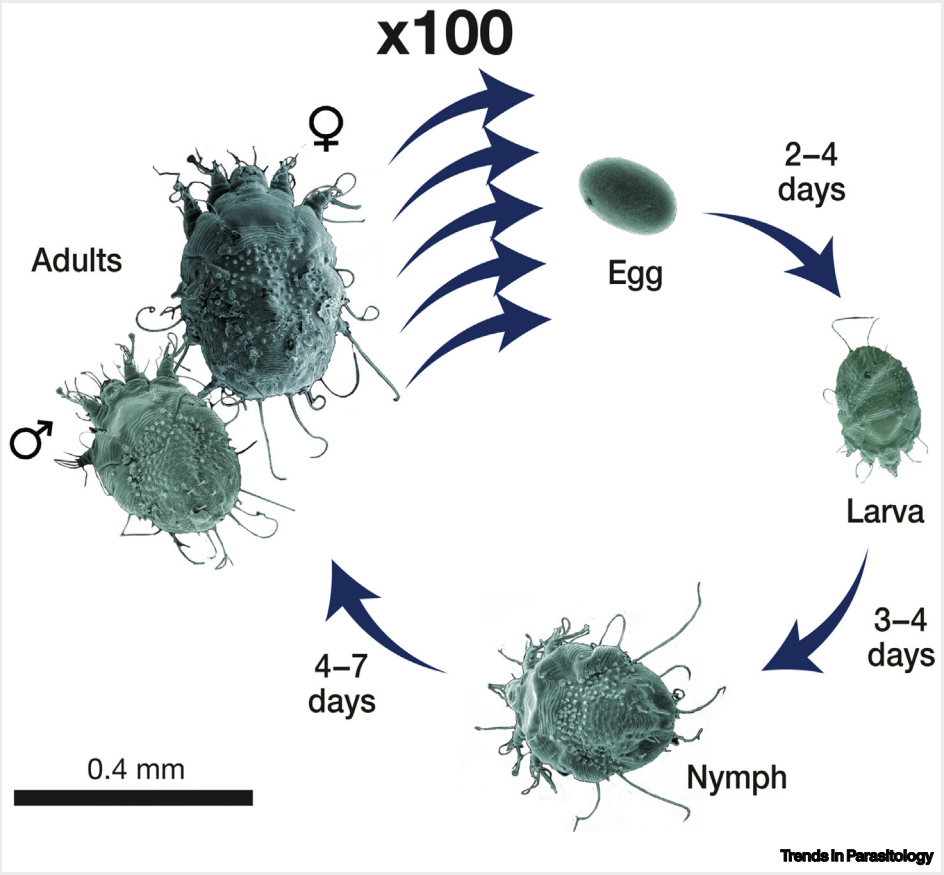


Figure I. Life Cycle of *Sarcoptes scabiei*. Scabies mites are obligately parasitic in their host's epidermis and undergo four distinct stages in a direct life cycle: egg, larva, nymphs, and adults. Adult female mites mate only once on the surface of the skin and remain fertile for the rest of their life (1–2 months). Impregnated females burrow into the skin and lay two or three eggs per day, up to 180 eggs in their lifetime. The eggs hatch as larvae within 2–4 days, and the six-legged larvae molt after 3–4 days into eight-legged protonymphs, then into slightly larger tritonymphs, and after 4–7 days, into adult mites. Scanning electron micrographs artificially colored.

Noncompliance with repeat treatments of both permethrin and ivermectin is a major complicating factor in intervention management at individual, household, and community levels, and can lead to treatment failure [13,14]. A further limitation of both drugs is that they are not suitable for all patients. Permethrin is not recommended for use in infants, and ivermectin is contraindicated in young children with a body weight of <15 kg, in pregnant or lactating women, and in patients with severely impaired liver or kidney function. Paradoxically, scabies incidence is highest in children of 1–24 months of age [15]. Apart from permethrin and ivermectin, only a few classical topical agents, such as sulfur (8–33%), crotamiton (10%), malathion (0.5%), and topical benzyl benzoate (10–25%) are presently available for use in children. However, the tolerability and clinical efficacies of these treatments have not been adequately assessed (see Table S1 in the supplemental information online). For example, although topical benzyl benzoate has rapid acaricidal action *in vitro*, it causes considerable skin irritation [16].

Current Scabies Treatments for Animals and Their Potential for Human Use

Different **biovars** of *S. scabiei* infect non-human mammals [17,18], causing sarcoptic mange and occasionally self-limiting lesions in humans [19,20]. Compared with the human situation, where there is a very limited number of acaricides, more compounds are available for the treatment of

Glossary

Bacteremia: a medical term referring to the presence of live bacteria within the blood.
Biovars: population variants of a species that differ physiologically and/or biochemically.
Blastoderm: the primary layer of cells surrounding the yolk resulting from nuclear divisions of the ovum. In many invertebrates, a syncytial blastoderm is initially formed and, subsequently, nuclei are partitioned into separate cells forming the cellular blastoderm around the yolk (blastula).
Disability-adjusted life years (DALYs): to compare health loss across different diseases, DALYs are a measure of disease burden and are calculated as the sum of the years of life lost (YLL) due to premature mortality and the years of life lived with disability.
Expressed sequence tag (EST): a fragment of a cDNA sequence, usually amplified from mRNA. As ESTs represent expressed genes in cells, tissues, or organs, they can be used to obtain data on gene expression or to identify and characterize expressed genes.
Formamidines: a group of insecticides for the control of Lepidoptera, Hemiptera, phytophagous mites, and some ticks. This group includes amitraz, chlordimeform, formetanate, formparanate, medimeform, and semiamitraz.
Gastrulation: following the blastula stage, cellular movements during this process result in the formation of the germ layers ectoderm, mesoderm, and endoderm. At the same time the segmentation starts.
Isoxazolines: a synthetic chemical class in veterinary products, such as afoxolaner (NexGard®), fluralaner (Bravecto®), lotilaner (Credelio®), and sarolaner (Simparica®), used to protect pets from fleas, ticks, or mites.
Macrocyclic lactones: contain two different subgroups, avermectins and milbemycins. They are products or chemical derivatives of soil microorganisms belonging to the genus *Streptomyces*. They have many broad-spectrum antiparasitic applications in veterinary medicine. The avermectins in commercial use are ivermectin, abamectin, doramectin, eprinomectin, and selamectin. The milbemycins (moxidectin and milbemycin) are chemically related to avermectins and

livestock and companion animals against sarcoptic mange [21] (Table 2). Moreover, most of the drugs that are used in humans were derived from the veterinary arsenal. The obvious gap between availability of human versus animal scabies treatment is mainly due to the companion animal and livestock health sector being a more lucrative market for industries than the **neglected tropical disease (NTD)** sector. Indeed, extensive research has led to the development of the new class of the **isoxazolines**, which appear to be highly effective against arthropods, including mites [22]. In animals, different formulations are used, including those for subcutaneous injection, oral products (tablets), and topical application: pour-on, spot-on, spray, or dip (listed in Table 2 and Table S1). Most of the drugs work on mite nerve axons by modifying the kinetics of ligand-gated ion channels [21]. The choice of compound usually depends on the animal species, the age of the animal, and the number of animals being treated. Because the parasite preferentially infects hard-to-treat body sites (e.g., inner-ear pinnae and vertical ear canal), and because of the thickness of the animal skin and density of fur, systemic treatments are often prioritized. Registered treatments for sarcoptic mange in dogs and livestock include **organophosphates**, **formamidines**, **pyrethroids**, **macrocyclic lactones**, and the very new isoxazolines (Table 2). Currently, the macrocyclic lactones are represented by seven developed drugs in two subfamilies: (i) avermectins, including ivermectin (for use in ruminants, horses, pigs, and humans), doramectin (ruminants and pigs), eprinomectin (cattle and cats), and selamectin (dogs and cats); (ii) milbemycins, including milbemycin oxime (dogs and cats), and moxidectin (ruminants, horses, dogs, and cats). Some of them can be combined with imidacloprid or an isoxazoline for the treatment and prevention of ectoparasites and nematodes [21]. Selamectin [23] and moxidectin, in combination with imidacloprid [24], are common treatments for sarcoptic mange in dogs and are given as a single spot-on treatment that should be repeated after 4 weeks. Because of treatment failures, veterinary dermatologists have recommended treatment at fortnightly intervals [24]. Ivermectin can be used off-label in dogs but is contraindicated in breeds such as Collies, Bearded Collies, Shetland Sheepdogs, Old English Sheepdogs, and Australian Shepherds because of a deletion mutation in the multidrug resistance gene (MDR1) linked to neurological ivermectin sensitivity [25].

Pyrethroids are synthetic acaricides which are derived structurally from the natural pyrethrins. Fenvalerate and deltamethrin are the two compounds that are used to treat ruminant livestock affected by sarcoptic mange. They are used as topical solutions diluted with water and applied directly to the skin of the animal; treatment is repeated after 10–12 days. Amitraz is a member of the formamidine family, used as an acaricide in veterinary practice. It can be used topically by washing animals (dogs, pigs, cattle, sheep, or goats), and needs to be repeated at weekly intervals until clinical signs of scabies disappear [26]. Amitraz is not approved for sarcoptic mange in all countries. Phoxim is an organophosphate that can be applied topically to pigs. Recently, isoxazolines have been developed as ectoparasiticides and can be used for the treatment of sarcoptic mange in dogs. Isoxazolines are a relatively new chemical class and include afoxolaner, fluralaner, sarolaner, and lotilaner [22]. They have a broad spectrum of insecticidal and acaricidal activities, and are effective against some ectoparasites, including fleas, ticks, and mites (i.e., *Demodex canis* and *S. scabiei*) [22,27,28]. Sarolaner was the first isoxazoline to be approved (November, 2015) for the indication of sarcoptic mange in dogs in most European countries, and more recently (December, 2018), afoxolaner and afoxolaner plus milbemycin oxime were also approved for the same indication. Only a few other agents appear to be efficient against *S. scabiei* in animals, among them Fipronil, which is a member of the phenylpyrazole family. It was reported in several therapeutic trials to be effective against canine scabies at a concentration of 0.25%, used as spot-on formulation at a dose of 3–6 ml/kg two to three times at weekly intervals, or as a spray [29,30]. All chemotherapies discussed here have been reported to work to some extent, but their efficacy is not well established.

have a similar mechanism of action and a longer half-life.
Multi-omics: this term refers to the use of multiple 'omic' (e.g., genomic, transcriptomic, and proteomic) methods to investigate the molecular biology of an organism, tissues, or cells.
Neglected tropical diseases (NTDs): a diverse group of diseases that are common in disadvantaged communities in developing countries of the subtropical and tropical regions of the world. They are caused by one or more pathogens, including viruses, bacteria, protozoa, helminths, or arthropods. NTDs are in contrast to known infectious diseases, such as HIV/AIDS, tuberculosis, and malaria, which usually receive greater attention and research funding.
Organophosphates: organophosphorus compounds that include the insecticides malathion, parathion, diazinon, fenthion, dichlorvos, chlorpyrifos, and ethion.
Pruritus: this term means 'itch' – a sensation that causes the desire or reflex to scratch.
Pyoderma: this term means any skin infection or disease that produces pus. Pyoderma can relate disorders such as impetigo, ecthyma, folliculitis, furuncle, and tropical ulcer.
Pyrethroids: pesticides that are similar to the natural pesticide pyrethrum produced by *Chrysanthemum* flowers, constituting most commercial household insecticides and insect repellents.
RNA interference (RNAi): a method that uses well-defined RNA molecules to silence or perturb genes.
Scabicides: medications used for treating scabies.
Septicemia: the presence of disease-causing bacteria or their toxins in the blood.
Stratum granulosum: from deep to superficial, the epidermal layers of the skin are the basal layer, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum. The stratum granulosum is primarily involved in providing waterproofing function. Keratinocytes within this layer generate the proteins keratin and keratohyalin.
Total RNA: all the RNA molecules inside a cell, including mRNA (long protein-coding mRNA transcripts), and small noncoding RNAs such as miRNA (genome-encoded negative regulators of translation and mRNA stability, modulating physiological and

The Egg Stage of *S. scabiei*

During oogenesis, a single germ cell undergoes several mitotic divisions to form a cell cluster (egg chamber) where all cells are in cytoplasmic connection with each other [31]. Among these cells, one single cell becomes the oocyte and other cells become the associated nurse cells, surrounding the oocyte. Adult females of *S. scabiei* mate only once and remain fertile for their life time (1–2 months) [32]. The male deposits sperm into the posterior cloaca of the female [33], which migrate up the genital tract to the ovaries where haploid oocytes are fertilized to produce (diploid) zygotes. Ovarian follicle cells surrounding the egg chamber secrete the vitelline membrane and the chorion, the outer layers of the subsequently formed embryo. Yolk is provided via lipids in the hemolymph. A shell gland located between the oviduct and vagina secretes substances that adhere to the embryo and produce the egg shell before oviposition. The eggs are laid through the 'tocostoma', a mid-ventral, transverse opening in the female mite [34], and then

developmental gene expression), and siRNA (small interfering RNAs processed from long double-stranded RNA), piRNA (PIWI-associated RNAs, another class of repressive small RNAs thought to protect the germline from mobile genome invaders such as transposons), and snoRNA [small nucleolar RNAs, a large conserved group of abundant small noncoding RNAs predominantly serving as guides for the chemical modification of ribosomal RNA (rRNA)].

Table 1. Comparison of Treatments in Use to Treat Scabies in Humans

Drugs	Formulations	Recommended treatments	Cost (Europe)	Efficacies (%)[a]	Main adverse reactions	Use in children	Use during pregnancy	Use in breastfeeding women
Ivermectin	200 µg/kg pills	Repeat after 7 days	€19 for 4 tablets at 3 mg = €38 for a complete treatment (weight 70 kg)	70–100	Nausea, rash, dizziness, itching, eosinophilia, abdominal pain, fever, tachycardia	Not approved in children <15 kg or 5 years of age	Only recommended in France	Only recommended in France
Permethrin	5% cream	Overnight – from head to toe – repeat once after 7–14 days	€19 for 30 g cream = €38 for a complete treatment	86–100	Pruritus, burning, stinging, eczema	Safe in children ≥2 months of age	Approved	Not recommended
Benzyl benzoate	10–25% lotion or emulsion	Apply from head to toe for 24 h on days 1, 2 and repeat after 7 days	€15 for 125 ml emulsion = €30 for a complete treatment	48–92	Pruritus, burning, stinging, pustules, skin irritation, eczema	Safe in children ≥1 month of age	Authorized if necessary	Not recommended
Crotamiton	10% cream	Overnight on days 1 and 2	€7 for 40 g cream	63–88	Pruritus, skin irritation, eczema, erythema, anaphylactic reaction	Safe in children	Not recommended	Not recommended
Precipitated sulfur	6–33% cream or lotion	Apply from head to toe for 3 consecutive nights	–	39–100	Messy application, malodor	Safe in children	Authorized	–
Malathion	0.5% aqueous lotion	Repeat after 7 days	€12 for 100 ml = €24 for a complete treatment	47–72	Pruritus, burning, stinging, skin irritation, central nervous system (CNS) toxicity, dizziness, seizure	Not approved in children <2 years of age	Not recommended	Not recommended
Lindane	1% lotion or cream	Overnight repeat after 7 days	–	64–96	CNS toxicity, dizziness, seizures, renal and hepatic toxicity reported with overdosage	Withdrawn from the European market	Withdrawn from the European market	Withdrawn from the European market

[a]Efficacies according to Strong and Johnstone (2007) [92].

Table 2. List of Drugs Registered for the Treatment of Sarcoptic Mange in Europe (May, 2019)

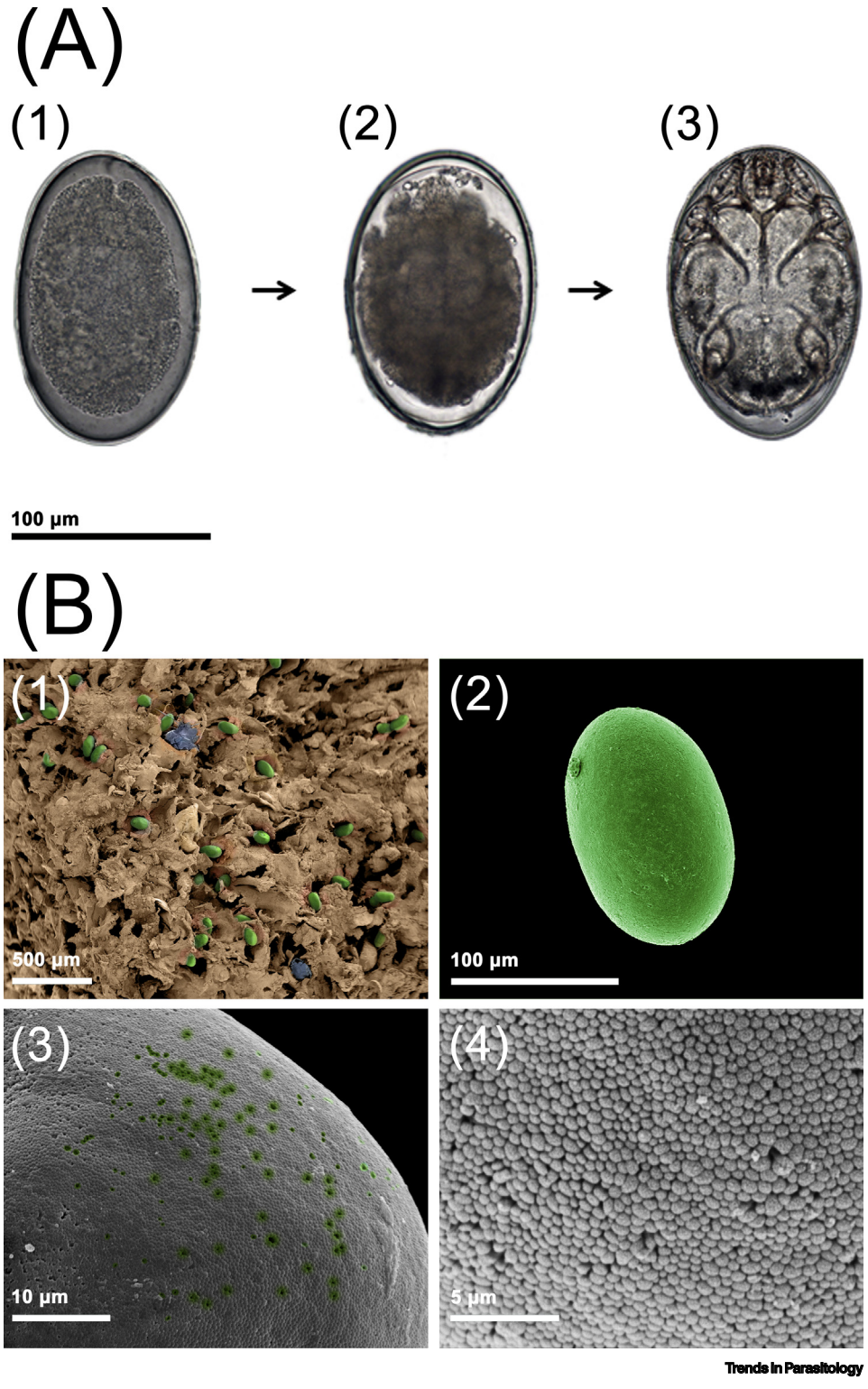
Family	Molecule	Brand name	Route of administration/ dosage	Animal species
Isoxazolines	Sarolaner	Simparica®	Tablet, 2–4 mg/kg	Dogs
	Afoxolaner	NexGard®	Tablet, 11.3–136 mg	Dogs
	Afoxolaner + milbemycin oxime	NexGard Spectra®	Tablet, 9/2mg–150/30 mg	Dogs
Macrocyclic lactones	Ivermectin	Ivomec®	Subcutaneous, pour on	Cattle, sheep, pigs
	Selamectin	Stronghold®	Spot-on, 6–10 mg/kg	Dogs
	Doramectin	Dectomax®	Subcutaneous	Cattle, sheep, pigs
		Doramec®	Subcutaneous	Cattle, sheep, pigs
	Eprinomectin	Eprinex®	Pour-on, 0.1 ml/kg	Cattle
	Moxidectin	Cydectin®	Pour-on, 0.5 mg/kg; SC, 0.2 mg/kg	Cattle, sheep
	Moxidectin + imidacloprid	Advocate®	Spot-on, 0.1 ml/kg	Dogs
	Milbemycin oxime	Interceptor®	Tablet, 1.0–1.5 mg/kg	Dogs
Pyrethroids	Fenvalerate	Acadrex®	Solution	Cattle
	Deltamethrin	Butox®	Solution 5%	Cattle, sheep
Formamidines	Amitraz	Taktic®	Solution 0.025–0.05%	Cattle, sheep, goats, pigs
Organophosphates	Phoxim	Sebacil®	Solution	Pigs

glued onto the burrow floor by an adhesive glycoprotein analogous to fibronectin [34], secreted by so-called 'glue glands'.

Mating is thought to occur in the moulting pouch of the female mite close to the host skin surface, and the fertilized female burrows into the skin where she remains for the rest of her life, laying eggs into her burrow as she extends it along the nutritious **stratum granulosum** layer [34,35] thus avoiding being eliminated by skin desquamation. It is estimated that a single adult female mite lays up to 180 eggs in her life time (Table S2). While eggs are the 'amplification stage' in the scabies life cycle (and should, as such, be eliminated by an effective drug), the number of eggs produced by parasitic mites is rather low compared with the high egg production seen in free-living arthropods (Table S2), whose eggs and hatching larvae are exposed to environmental challenges such as predators and climate.

The eggs of *S. scabiei* are oval and ~0.15 mm in length (Figure 1). The egg shell, also known as the 'choroid membrane', consists of two distinct layers: the outermost papillary layer (Figure 1B) and the basement layer. The papillary layer consists of closely grouped papillae-like structures (0.9 µm in length), with a pentagonal or hexagonal shaped surface [34,36] (Figure 1B). The basement layer of the shell is a homogeneous sheath (0.5–0.8 µm thick). It consists of electron-translucent material flanked by an electron-dense coat [36]. Micropores, which appear irregularly throughout the choroid layer (Figure 1B), are believed to act as aeropyles [36], a system of air ducts within the chorion, enabling continuous gas exchange with the environment. Beneath the egg shell are two extra embryonic envelopes, the vitelline membrane and the chorion, which directly surround the embryo.

Eggs of *S. scabiei* hatch 48–96 h after oviposition. Although egg development can be monitored by light microscopy (Figure 1A), *S. scabiei* embryogenesis is largely unknown. As established for other mites [37], it is thought that the diploid zygote undergoes several mitotic divisions to produce 4, 8, 16, 32, 64, and 128 nuclei in early cleavage stages. Subsequently, the superficial **blastoderm** forms an outer cell lining just below the egg shell surrounding the yolk. **Gastrulation**



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begins when a funnel-shaped invagination appears at one end of the egg. Subsequently, the so-called germ-bands connect this funnel and the superficial cell layers. The next cleavage stages give rise to a longitudinal furrow, which defines the anterior and posterior axis of the embryo. Several transverse furrows are formed, which divide the paired buds of prosomal limbs. Differentiation of appendages proceeds from front to back. The posterior part (opisthosoma) of the body begins to develop as the body of the embryo grows within the oblong egg. Immediately prior to hatching, a completely developed larva is visible inside the egg (Figure 1A).

Eggs are very different from the motile stages, and identifying specific morphological features in the egg could aid in understanding why this particular stage is not affected by most of the drugs presently in use. The protective, thick egg shell, composed of two distinct intimately connected layers [36], may be a first challenge for a drug to reach its target, and it may be impermeable for currently used drugs. The egg stage can be described as two phenotypes: (i) an early embryonic neurologically immature and immobile accumulation of cells with an absence of a differentiated nervous system, and (ii) a mature embryo, still inside the egg, with the same vital systems found in newly emerged larva, including the nervous, respiratory, and circulatory systems. Accordingly, there might be two ways of targeting the egg: (i) killing the developing early stage embryo, or (ii) killing the developed larva when it comes into contact with the surface of an egg shell impregnated with an acaricide during hatching. The early embryonic stage appears to be unexplored in terms of drug targets. Targeting the motile larva within a mature egg should be possible with a neurotoxin, but the half-life of a drug in skin would need to be >3 days. Another option may be to target the egg-hatching process itself, as it is thought to be a complex multistaged event, involving enzymes and mechanical cleavage of the egg shell [38,39].

Thus, an effective ovicidal drug might need to have at least one of several modes of action. It might target early embryonic development, with the capacity to penetrate the protective egg shell and extraembryonic envelopes, or target vital processes common to mature embryos and motile forms, or interfere with hatching. The later it interferes in egg development, the longer it must remain active in the skin. Following this line of thought, we looked for compounds that are ovicidal for other arthropods and might be applicable to mites.

Ovicidal Treatments against Other Arthropods

There is an overwhelming diversity in the morphology and lifecycles of arthropods, reflected in an impressive array of embryologies. Even within the Acari (mites and ticks), the embryonic development of ticks is distinctly different from that of mites [40]. Keeping this in mind, we reviewed the literature for drugs targeting the embryos of arthropods (Table S3), to aid in identifying and predicting drugs that might be ovicidal to the eggs of *S. scabiei*.

Most insecticides currently in use are either larvicidal or adulticidal, but very few are ovicidal. Typically, the mode of action of these drugs and the embryonic stage affected by the drug are unclear. Interestingly, some available drugs sterilize the female arthropod and/or affect the growth of the egg. For example, abamectin (a macrocyclic lactone) substantially affects the reproductive system of *Solenopsis invicta* (fire ant), leading to sterility of the queen, and also causes reduced egg production, size, and yolk [41].

Figure 1. The *Sarcoptes scabiei* Egg Stage. (A) Embryo development within the egg, shown by light microscopy. (1) Newly laid egg. (2) Egg with partially developed larva. (3) Egg with larva about to hatch. Nikon SMZ645, ×40. Courtesy of Dr Fang, Parasitology EnvA, Maisons-Alfort, France. (B) Scanning electron microscopy of *S. scabiei* eggs. (1) Eggs (artificially colored green, magnification ×44) deposited within porcine skin crusts. (2) Egg artificially colored green, magnification ×300. (3) Outermost papillary layer showing distinct pores (artificially colored green, magnification ×2700). (4) Close view of the egg surface featuring papillary layer and micropores (magnification ×6000).

Almost all of the prevailing drugs are neurotoxins. Strategies to combine two or more compounds with different properties and targets, to obtain a synergic or additive activity, have been realized for the treatment of fleas in companion animals [37–39].

Discovering New Intervention Targets with Advanced Technologies

Drug discovery is far less advanced for mites than it is for other parasites such as worms. As for other pathogens, indications for novel targets might come from mining **multi-omic** databases. Mining approaches should aim for extracellular scabies mite molecules that ideally (i) have activity that can be modulated by a drug, (ii) are unique and essential to life and survival, or (iii) underpin reproductive or disease processes.

S. scabiei is challenging to study at the molecular level because it is an obligate parasite. The mites are microscopic, and most patients presenting with classical scabies have low mite burdens (<20 mites). Until the early 2000s, almost no experimental and molecular data existed on scabies mites due to the absence of *in vitro* culture and an animal model. Since then, some essential molecular databases have been established, including a human scabies mite **expressed sequence tag (EST)** dataset (reviewed in [42]), from which proteins implicated in allergy, drug resistance, and immune evasion were identified, in addition to candidate drug and immunodiagnostic targets. Indeed, the first biologically active recombinant molecules representing multiple classes of scabies mite proteins were produced and some of their roles in pathogenicity and mite survival were characterized [43,44]. Compared with ticks, mites have substantially smaller genomes [45], and as genomic technologies have advanced, and sequencing costs declined, *de novo* assembly of the scabies mite draft nuclear [46] and mitochondrial [47] genomes has become a reality.

Advances in nucleic acid sequencing and bioinformatics technologies have enabled an unprecedented number of arthropod genomes to be decoded (e.g., [48,49]). While draft genomes provide investigators with resources to explore arthropods at the molecular level, the transcription/expression profiles and functions of most genes of acarines, including mites and ticks, are largely unknown. Some researchers have begun to use genome sequences to assist in studying the expression, localization, and function of genes employing RNA sequencing [50,51] and proteomics tools [52], but this field is still in its infancy.

RNA (transcriptomic) sequencing quantifies particular types of transcripts, including **total RNA**, polyadenylated RNA, and small RNAs from whole arthropods, or specific developmental stages or tissues. Proteomics provides a means of identifying proteins in the arthropods. Importantly, resources to explore and mine such arthropod data sets are now available, for example, the International Nucleotide Sequence Database Collaboration (INSDC) database[i], InsectBase[ii], and FlyBase[iii] provide a wealth of functional and structural genomic information.

Clearly, some genome-sequencing projects have produced significant amounts of data for acarines, including mites [46,53–56]. However, a critical analysis of the literature reveals that (i) most genomes are drafts and, thus, are fragmented; (ii) only a small part of a genome codes for proteins; (iii) a large number of proteins encoded in the genome are orphans (unknown); and (iv) most of the genome is DNA that we know nothing about (and is thus called 'dark matter'), but we expect that this matter will have crucial functional and regulatory roles [57]. Findings from the model organism Encyclopedia of DNA Elements (modENCODE) project [58] would indicate highly significant functional and regulatory elements that control the expression of hundreds of genes and, consequently, influence the physiology, biochemistry, and behavior (phenotype) of arthropods, such as mites, and their tissues and cells. Although technically challenging, exploring

the structure and function of unique genes/gene families in *S. scabiei* and related mites could identify essential molecular biological pathways linked to parasitism and the pathogenesis of scabies.

A prerequisite for such molecular work, though, will be to sequence the genomes of representative biovars of *S. scabiei* (e.g., from human, pig, and canids) to chromosome-scale contiguity. To do this, an automated bioinformatic pipeline could be used to assemble both long- and short-read sequence data sets [59], and accurately predict the genes [60]. Using complete mite genomes, tandem multigene families that are crucial to understanding *S. scabiei*-specific traits of biological importance (e.g., adaptation, parasitism, fitness, virulence, pathogenicity, and drug resistance) could be inferred and characterized.

Genomic and transcriptomic comparisons between both parasitic and nonparasitic mite species should allow the identification of genes undergoing positive selection or gene family amplification within parasitic lineages and are central to parasitism. In addition to gaining a deep understanding of genes that are differentially transcribed between/among developmental stages, it might be possible to explore how the transcriptome of *S. scabiei* is regulated via large-scale comparisons of conserved noncoding DNA with possible regulatory functions. This focus might enable the discovery of a means of blocking expression of coregulated gene families (e.g., those encoding complement-inhibiting SMIPP-Ss [61]) whose collective activity might be responsible for promoting infestation, parasitism, and/or disease.

Given that gene silencing has been shown to work in *S. scabiei* [62], the functionality of orphan molecules could be explored by double-stranded **RNA interference (RNAi)** [63] or theoretically by knockout using a clustered regularly interspaced short palindromic repeats (CRISPR)-based approach [64]. Panels of *S. scabiei*-specific genes could be tested, and high-resolution imaging used to identify non-wild-type phenotypes (e.g., motility, lethality, or developmental defects) and confirm knock-down specificity by transcription analysis (RNA-sequencing) and proteomics. Structural or morphological alterations in perturbed mites could be assessed by advanced imaging. Such a focus could provide an avenue to identify and characterize essential, *S. scabiei*-specific genes as drug targets. It might also be possible to comprehensively define miRNAs and other noncoding RNAs encoded in the *S. scabiei* genome, and determine a subset of noncoding RNAs that contribute directly to parasitism. This would be important for understanding the obligatory parasitic mode of existence of *S. scabiei*; distinct developmental stages of this mite are proposed to secrete miRNAs, some of which likely exert immunomodulatory effects on their mammalian hosts, and/or govern the host–parasite interplay [65]. Although these are challenging areas to investigate for a mite as tiny and as complex in biology as *S. scabiei*, advances here could lead to a significant shift in our understanding of scabies at the molecular level, which would likely enable the discovery and development of new interventions.

Evaluating a New Treatment Using an Established Pig-Scabies Model

Of major importance for all of the aforementioned research is unconstrained access to all developmental stages of *S. scabiei*, which is possible through an experimental porcine model for human scabies [66]. Although some physiological differences may determine host preferences, no unequivocal morphological differences exist between *S. scabiei* biovars from human and porcine hosts [67], immunological cross reactivity has been demonstrated for multiple proteins (reviewed in [68]), and recent mitochondrial genome sequencing data indicate that some porcine and human biovars are genetically closely related [47]. Importantly, pigs and humans exhibit a very similar skin physiology [69] and clinical manifestation of *S. scabiei* infestation [70]. Hence, the use of the pig-*S. scabiei* infestation model appears to be an appropriate system for the critical

evaluation of new drugs, including absorption, distribution, metabolism, excretion, and toxicity (ADMET) studies, prior to clinical trials in humans. This statement is supported somewhat by two recent preclinical trials using this model, demonstrating that single oral treatments with either moxidectin [10] or afoxolaner [71] were superior to two oral-dose treatments with ivermectin. Based on this work, industry has now commenced investment in anti-scabies treatments. For example Medicines Development for Global Health, a not-for-profit biopharmaceutical company, has raised A$3 million for a key Phase II trial (NCT03905265) to assess moxidectin to treat human scabies, the first and largest clinical venture in this area.

Concluding Remarks

Globally, scabies prevalence remains constant or is rising, clinical evidence of the limitations of currently available treatments is mounting, and drug resistance is developing in the parasite. Novel drugs would be a significant improvement of this dire situation. There is a range of research activities pending in this field (see Outstanding Questions). Novel scabicide targets and candidates discovered through biomolecular research of the parasite, or via knowledge transfer from the veterinary drug arsenal and validated in preclinical *in vivo* studies, are all plausible, but have not yet been tackled. It would be desirable to obtain critical knowledge concerning the mode of action of candidate drugs via elucidation of molecular pathways and/or biological targets in the mite itself. Targeting the eggs and adult stages of *S. scabiei* seems to be a sound approach, which, to our knowledge, has not yet been proposed. A treatment that completely breaks the life cycle would need to be administered only once, and noncompliance to repeat treatments would not be an issue. Such a single-dose treatment would likely substantially improve the clinical management of scabies in its challenging settings. This treatment strategy, and the approaches developed for scabies, could have broader implications for the management or control of other arthropod-associated diseases worldwide.

Outstanding Questions

Which drugs that have been used successfully for the treatment of ectoparasites could be applied to scabies?

Which drug targets are critical for survival and life of *S. scabiei* throughout its life cycle?

Which molecules or molecular pathways in *S. scabiei* are best targeted for the design of new and effective scabicides?

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Supplemental Information

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Resources

[i]http://i5k.github.io/arthropod_genomes_at_ncbi

[ii]www.insect-genome.com/

[iii]https://flybase.org/

References

1. Liu, X. *et al.* (2014) Vaccine against scabies: necessity and possibility. *Parasitology* 141, 725–732
2. Chosidow, O. (2006) Scabies. *N. Engl. J. Med.* 354, 1718–1727
3. Strong, M. and Johnstone, P. (2007) Interventions for treating scabies (Review). *Cochrane Database Syst. Rev.* 3
4. Rosumeck, S. *et al.* (2018) Ivermectin and permethrin for treating scabies. *Cochrane Database Syst. Rev.* 4
5. Wolstenholme, A.J. and Rogers, A.T. (2005) Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. *Parasitology* 131, S85–S95

6. Wolstenholme, A.J. (2012) Glutamate-gated chloride channels. *J. Biol. Chem.* 287, 40232–40238
7. Mounsey, K.E. *et al.* (2008) Scabies: molecular perspectives and therapeutic implications in the face of emerging drug resistance. *Future Microbiol.* 3, 57–66
8. Romani, L. *et al.* (2015) Mass drug administration for scabies control in a population with endemic disease. *N. Engl. J. Med.* 373, 2305–2513
9. Steer, A.C. *et al.* (2009) Ivermectin worthy of further investigation. *Bull. World Health Org.* 87, A-A
10. Bernigaud, C. *et al.* (2016) Preclinical study of single-dose moxidectin, a new oral treatment for scabies: efficacy, safety, and pharmacokinetics compared to two-dose ivermectin in a porcine model. *PLoS Negl. Trop. Dis.* 10, e0005030
11. Mounsey, K.E. *et al.* (2017) *In vitro* efficacy of moxidectin versus ivermectin against *Sarcoptes scabiei*. *Antimicrob. Agents Chemother.* 61, 8
12. Mounsey, K.E. *et al.* (2009) Longitudinal evidence of increasing *in vitro* tolerance of scabies mites to ivermectin in scabies-endemic communities. *Arch. Dermatol.* 145, 840–841
13. Aussy, A. *et al.* (2019) Risk factors for treatment failure in scabies: a cohort study. *Br. J. Dermatol.* 180, 888–893
14. Williams, C. and Fuller, L.C. (2019) Why does scabies treatment fail? *Br. J. Dermatol.* 180, 710–711
15. Landwehr, D. *et al.* (1998) Epidemiologic aspects of scabies in Mali, Malawi, and Cambodia. *Int. J. Dermatol.* 37, 588–590
16. Hogan, D.J. *et al.* (1991) Diagnosis and treatment of childhood scabies and pediculosis. *Pediatr. Clin. N. Am.* 38, 941–957
17. Fraser, T.A. *et al.* (2016) The emergence of sarcoptic mange in Australian wildlife: an unresolved debate. *Parasit. Vectors* 9, 316–327
18. Scott, D. *et al.* (2004) Sarcoptic mange in raccoons in Michigan. *J. Wildl. Dis.* 40, 3
19. Bandi, K.M. and Saikumar, C. (2013) Sarcoptic mange: a zoonotic ectoparasitic skin disease. *J. Clin. Diagnost. Res.* 7, 156–157
20. Beck, A.L. (1965) Animal scabies affecting man. *Arch. Dermatol.* 91, 54–55
21. Beugnet, F. and Franc, M. (2012) Insecticide and acaricide molecules and/or combinations to prevent pet infestation by ectoparasites. *Trends Parasitol.* 28, 267–279
22. Weber, T. and Selzer, P.M. (2016) Isoxazolines: a novel chemotype highly effective on ectoparasites. *ChemMedChem* 11, 270–276
23. Six, R.H. *et al.* (2000) Efficacy and safety of selamectin against *Sarcoptes scabiei* on dogs and *Otodectes cynotis* on dogs and cats presented as veterinary patients. *Vet. Parasitol.* 91, 291–309
24. Fourie, L.J. *et al.* (2006) The efficacy of an imidacloprid/moxidectin combination against naturally acquired *Sarcoptes scabiei* infestations on dogs. *Aust. Vet. J.* 84, 17–21
25. Menez, C. *et al.* (2012) Relative neurotoxicity of ivermectin and moxidectin in Mdr1ab (–/–) mice and effects on mammalian GABA(A) channel activity. *PLoS Negl. Trop. Dis.* 6, e1883
26. Johansson, L.E. *et al.* (1980) Amitraz–(TAKTIC) for the control of pig mange. *Nord. Vet. Med.* 32, 161–164
27. Beugnet, F. *et al.* (2016) Efficacy of afoxolaner in a clinical field study in dogs naturally infested with *Sarcoptes scabiei* (Efficacité de l'afoxolaner dans une étude de terrain clinique, chez des chiens infestés naturellement par *Sarcoptes scabiei*). *Parasite (Paris)* 23, 26–33
28. Becskei, C. *et al.* (2016) Efficacy and safety of a novel oral isoxazoline, sarolaner (Simparica), for the treatment of sarcoptic mange in dogs. *Vet. Parasitol.* 222, 56–61
29. Bordeau, W. and Hubert, B. (2000) Treatment of 36 cases of canine *Sarcoptes* using a 0.25% fipronil solution. *Vet. Dermatol.* 11, 27
30. Curtis, C.F. (1996) Use of 0.25 per cent fipronil spray to treat sarcoptic mange in a litter of five-week-old puppies. *Vet. Rec.* 139, 43–44
31. Slack, J.M.W. (1991) *From Egg to Embryo*, Cambridge University Press
32. Heilesen, B. (1946) *Studies on Acarus scabiei and Scabies*, Rosenkilde & Bagger
33. Desch, C.E. (2001) Anatomy and ultrastructure of the female reproductive system of *Sarcoptes scabiei* (Acari: Sarcoptidae). Acarology. In *Proceedings of the 10th International Congress*, pp. 203–212
34. Shelley, W.B. and Shelley, E.D. (1983) Scanning electron microscopy of the scabies burrow and its contents, with special reference to the *Sarcoptes scabiei* egg. *J. Am. Acad. Dermatol.* 9, 673–679
35. Banzhaf, C.A. *et al.* (2013) *In vivo* imaging of *Sarcoptes scabiei* infestation using optical coherence tomography. *Case Rep. Dermatol.* 5, 156–162
36. Mazzini, M. and Baiocchi, R. (1983) Fine morphology of the eggshell of *Sarcoptes scabiei* (L.) (Acarina: Sarcoptidae). *Int. J. Parasitol.* 13, 469–473
37. Chetverikov, P.E. and Desnitskiy, A.G. (2016) A study of embryonic development in eriophyoid mites (Acariformes, Eriophyoidea) with the use of the fluorochrome DAPI and confocal microscopy. *Exp. Appl. Acarol.* 68, 97–111
38. Van Neste, D. *et al.* (1981) Life cycle of scabies mite (*Sarcoptes scabiei* var. *hominis*) studied by scanning electron microscopy. *Ann. Dermatol. Venereol.* 108, 355–361
39. Holmes, S.D. and Fairweather, I. (1982) *Hymenolepis diminuta*: the mechanism of egg hatching. *Parasitology* 85, 237–250
40. Santos, V.T. *et al.* (2013) The embryogenesis of the tick *Rhipicephalus* (*Boophilus*) *microplus*: the establishment of a new chelicerate model system. *Genesis* 51, 803–818
41. Lasota, J.A. and Dybas, R.A. (1991) Avermectins, a novel class of compounds: implications for use in arthropod pest control. *Annu. Rev. Entomol.* 36, 91–117
42. Fischer, K. *et al.* (2012) Scabies: important clinical consequences explained by new molecular studies. *Adv. Parasitol.* 79, 339–373
43. Beckham, S.A. *et al.* (2009) Characterization of a serine protease homologous to house dust mite group 3 allergens from the scabies mite *Sarcoptes scabiei*. *J. Biol. Chem.* 284, 34413–34422
44. Bergstrom, F.C. *et al.* (2009) Scabies mite inactivated serine protease paralogs inhibit the human complement system. *J. Immunol.* 182, 7809–7817
45. Mounsey, K.E. *et al.* (2012) Quantitative PCR-based genome size estimation of the astigmatid mites *Sarcoptes scabiei*, *Psoroptes ovis* and *Dermatophagoides pteronyssinus*. *Parasit. Vectors* 5, 3
46. Mofiz, E. *et al.* (2016) Genomic resources and draft assemblies of the human and porcine varieties of scabies mites, *Sarcoptes scabiei* var. *hominis* and var. *suis*. *Gigascience* 5, 23
47. Mofiz, E. *et al.* (2016) Mitochondrial genome sequence of the scabies mite provides insight into the genetic diversity of individual scabies infections. *PLoS Negl. Trop. Dis.* 10, e0004384
48. Gonzalez, V.L. *et al.* (2018) Open access genomic resources for terrestrial arthropods. *Curr. Opin. Insect. Sci.* 25, 91–98
49. Poelchau, M.F. *et al.* (2018) Navigating the i5k Workspace@NAL: a resource for arthropod genomes. *Methods Mol. Biol.* 1757, 557–577
50. Zhang, J. *et al.* (2017) Differential gene expression in *Anopheles stephensi* following infection with drug-resistant *Plasmodium yoelii*. *Parasit. Vectors* 10, 401
51. Bonizzoni, M. *et al.* (2015) RNA-seq analyses of changes in the *Anopheles gambiae* transcriptome associated with resistance to pyrethroids in Kenya: identification of candidate-resistance genes and candidate-resistance SNPs. *Parasit. Vectors* 8, 474
52. Hugo, R.L.E. and Birrell, G.W. (2018) Proteomics of *Anopheles* vectors of malaria. *Trends Parasitol.* 34, 961–981
53. Grbic, M. *et al.* (2011) The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature* 479, 487–492
54. Chan, T.F. *et al.* (2015) The draft genome, transcriptome, and microbiome of *Dermatophagoides farinae* reveal a broad spectrum of dust mite allergens. *J. Allergy Clin. Immunol.* 135, 539–548
55. Burgess, S.T.G. *et al.* (2018) Draft genome assembly of the sheep scab mite, *Psoroptes ovis*. *Genome Announc.* 6, 16
56. Rider, S.D. *et al.* (2015) Draft genome of the scabies mite. *Parasit. Vectors* 8, 585
57. St Laurent, G. *et al.* (2012) Dark matter RNA: an intelligent scaffold for the dynamic regulation of the nuclear information landscape. *Front. Genet.* 3, 57

58. Brown, J.B. and Celniker, S.E. (2015) Lessons from modENCODE. *Annu. Rev. Genomics Hum. Genet.* 16, 31–53
59. Korhonen, P.K. *et al.* (2019) Common workflow language (CWL)-based software pipeline for *de novo* genome assembly from long- and short-read data. *Gigascience* 8, 4
60. Korhonen, P.K. *et al.* (2016) Making sense of genomes of parasitic worms: Tackling bioinformatic challenges. *Biotechnol. Adv.* 34, 663–686
61. Reynolds, S.L. *et al.* (2014) Scabies mite inactive serine proteases are potent inhibitors of the human complement lectin pathway. *PLoS Negl. Trop. Dis.* 8, e2872
62. Fernando, D.D. *et al.* (2017) Gene silencing by RNA interference in *Sarcoptes scabiei*: a molecular tool to identify novel therapeutic targets. *Parasit. Vectors* 10, 289
63. Niu, J. *et al.* (2018) Beyond insects: current status and achievements of RNA interference in mite pests and future perspectives. *Pest Manag. Sci.* 74, 2680–2687
64. Bier, E. *et al.* (2018) Advances in engineering the fly genome with the CRISPR-Cas system. *Genetics* 208, 1–18
65. He, R. *et al.* (2017) Transcriptome-microRNA analysis of *Sarcoptes scabiei* and host immune response. *PLoS One* 12, e0177733
66. Mounsey, K. *et al.* (2010) A tractable experimental model for study of human and animal scabies. *PLoS Negl. Trop. Dis.* 4, e756
67. Fain, A. (1978) Epidemiological problems of scabies. *Int. J. Dermatol.* 17, 20–30
68. Arlian, L.G. and Morgan, M.S. (2017) A review of *Sarcoptes scabiei*: past, present and future. *Parasit. Vectors* 10, 297–319
69. Meurens, F. *et al.* (2012) The pig: a model for human infectious diseases. *Trends Microbiol.* 20, 50–57
70. Van Neste, D.J. and Staquet, M.J. (1986) Similar epidermal changes in hyperkeratotic scabies of humans and pigs. *Am. J. Dermatopathol.* 8, 267–273
71. Bernigaud, C. *et al.* (2018) Efficacy and pharmacokinetics evaluation of a single oral dose of Afoxolaner against *Sarcoptes scabiei* in the porcine scabies model for human infestation. *Antimicrob. Agents Chemother.* 62, e02334-17
72. Lynar, S. *et al.* (2017) Scabies and mortality. *Lancet Infect. Dis.* 17, 1234
73. Chosidow, O. and Hay, R.J. (2019) Control of scabies and secondary impetigo: optimising treatment effectiveness in endemic settings. *Lancet Infect. Dis.* 19, 454–456
74. Hay, R.J. (2003) Pyoderma and scabies: a benign association? *Curr. Opin. Infect. Dis.* 16, 69–70
75. Bhat, S.A. *et al.* (2017) Host immune responses to the itch mite, *Sarcoptes scabiei*, in humans. *Parasit. Vectors* 10, 385–397
76. Kemp, D.J. *et al.* (1999) Crusted scabies: a molecular analysis of *Sarcoptes scabiei* variety *hominis* populations from patients with repeated infestations. *Clin. Infect. Dis.* 29, 1226–1230
77. Walton, S.F. *et al.* (2008) New insights into disease pathogenesis in crusted (Norwegian) scabies: the skin immune response in crusted scabies. *Br. J. Dermatol.* 158, 1247–1255
78. Chang, A.Y. and Fuller, L.C. (2018) Scabies – an ancient disease with unanswered questions in modern times. *JAMA Dermatol.* 154, 999–1000
79. Romani, L. *et al.* (2015) Prevalence of scabies and impetigo worldwide: a systematic review. *Lancet Infect. Dis.* 15, 960–967
80. Roberts, L.J. *et al.* (2005) Crusted scabies: clinical and immunological findings in seventy-eight patients and a review of the literature. *J. Infect.* 50, 375–381
81. Walton, S.F. (2010) The immunology of susceptibility and resistance to scabies. *Parasite Immunol.* 32, 532–540
82. Hay, R.J. *et al.* (2012) Scabies in the developing world-its prevalence, complications, and management. *Clin. Microbiol. Infect.* 18, 313–323
83. Cassell, J.A. *et al.* (2018) Scabies outbreaks in ten care homes for elderly people: a prospective study of clinical features, epidemiology, and treatment outcomes. *Lancet Infect. Dis.* 18, 894–902
84. Monro, I.C. (1942) Spread of scabies by fomites. *Br. Med. J.* 1, 803–804
85. Karimkhani, C. *et al.* (2017) Global skin disease morbidity and mortality: an update from the Global Burden of Disease Study 2013. *JAMA Dermatol.* 153, 406–412
86. Fuller, L.C. (2013) Epidemiology of scabies. *Curr. Opin. Infect. Dis.* 26, 123–126
87. Karimkhani, C. *et al.* (2017) The global burden of scabies: a cross-sectional analysis from the Global Burden of Disease Study 2015. *Lancet Infect. Dis.* 17, 1247–1254
88. Walton, S.F. and Currie, B.J. (2007) Problems in diagnosing scabies, a global disease in human and animal populations. *Clin. Microbiol. Rev.* 20, 268–279
89. Engelman, D. *et al.* (2016) Opportunities for Integrated control of neglected tropical diseases that affect the skin. *Trends Parasitol.* 32, 843–854
90. WHO (2017) *Report of the Tenth Meeting of the WHO Strategic and Technical Advisory Group for Neglected Tropical Diseases 29–30 March 2017*, WHO
91. Mounsey, K.E. *et al.* (2013) Scratching the itch: new tools to advance understanding of scabies. *Trends Parasitol.* 29, 35–42
92. Strong, M. and Johnstone, P. (2007) Interventions for treating scabies. *Cochrane Database Syst. Rev.* 3, CD000320