Immunocontraception as a possible tool to reduce feral pig populations: recent and future perspectives

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Abstract

The feral pig populations of many countries continue to increase. Scientific studies on population size are scarce, while the numbers of reported observations on presence of and damage caused by feral pigs are increasing. Feral pigs can carry and spread several diseases (including zoonotic), but African Swine Fever (ASF) is of most concern. It is a highly transmissible viral disease associated with an extremely high mortality rate. Since 2009 ASF has appeared in several European countries, with cases being identified first among local feral pigs and consequently in domestic pig production units, indicating a clear linkage with the movement of the feral pig population and the spread of the disease across national boundaries. Control of feral pig populations is currently under discussion. Because massive culling raises questions of animal welfare and ethics, fertility control could represent an important and effective means to control feral pig populations. Contraceptive vaccines have been used with some degree of success in many wild species because they are able to provide a long-term effect without any consequent health problems. However, extensive and efficacious use of vaccines to control feral pig populations is not simple. The aim of this article was to review the progress in immunocontraception use in feral pigs, providing an account of the current status and future perspectives.

Introduction

Populations of feral pigs have been increasing during the last 20 years (Tack, 2018). Because feral pigs reproduce intensively (Fernández-Llario, 2004), live isolated and largely nocturnal lives (Lemel et al., 2003) and are able to migrate over long distances, it is very difficult to estimate population sizes accurately (Vetter et al., 2015). In the state of Texas the number of feral pigs is reported to range from 1 to 4 million (Timmons et al., 2012). Although not based on scientific studies, the number of observations and increase in reported damage suggest that the feral pig population is continuously growing and expanding (Timmons et al., 2012). Ongoing increase in the feral pig population is also apparent in Europe (Massei et al., 2015). Recent reports show that the number of harvested feral pigs has consistently increased during the past decade throughout many European countries (Tack, 2018), while the number of hunters has remained relatively stable or declined in most countries (Massei et al., 2015). Increasing incidence of mild winters, intensification of arboreal growth in forest areas, crop production, and compensatory population responses of feral pigs to hunting pressure might also explain their population growth (Massei et al., 2015). Other than man, the most significant contributor to its mortality (primarily through hunting or car accidents) (Morelle et al., 2013; Šprem et al., 2013), the feral pig has few predators. The grey wolf is considered to be its main predator: a single wolf can kill around 50–80 feral pigs in a year (Heptner et al., 1988), and in National Park areas of Italy and Belarus feral pigs are the wolf's primary prey (Marsan & Mattioli, 2013). Another issue of increasing conflict between feral pigs and humans is damage to agricultural crops (Bleier et al., 2012; Lombardini et al., 2017). Timmons et al. (2012) reported that agriculture losses due to feral pigs are at least $52 million each year solely in the state of Texas. Moreover, feral pigs in most areas are not among the native species, may serve as a vehicle of transmission for infectious diseases to humans and domestic pigs. Swine hepatitis E virus (HEV) is a zoonotic agent and bears a close genomic resemblance to human HEV. The disease causes asymptomatic infection in swine and is a public health concern, causing acute hepatitis of varying severity in humans (Salines et al., 2017). Classical swine fever (CSF), which is still one of the main viral diseases of pigs (Pejsak et al., 2014), can be spread by feral pigs acting as the reservoir for the virus, as reported in Europe (Tack, 2018). Many other zoonotic diseases can affect feral pigs, including Pasteurellosis, hemorrhagic septicemia, tularemia, anthrax and Brucella, and they can be carriers of several endo- and ectoparasites (Tack, 2018). Among all the diseases that can be spread by feral pigs, African Swine Fever (ASF), which is a highly transmissible viral disease associated with an extremely high mortality rate (Galindo & Alonso, 2017), is of greatest concern. Starting from 2009 ASF has appeared in several European countries, firstly in local feral pigs and consequently in production units of domestic pigs, showing a clear linkage with the movement of the feral pig population and the spread of the disease between countries (Guinat et al., 2016b; Galindo & Alonso, 2017). Due to sanitary legislation, when ASF is confirmed in a particular country it heavily affects the pork industry, causing huge losses of animals due enforced culling, mortality of infected animals and severe trade restrictions (Guinat et al., 2016a). It is clear that in many areas the feral pig population is reaching a level which increases conflicts with humans, poses an increasing risk for animal and human health, and possibly becomes a serious threat to the pork industry. This explains why control of feral pig populations is currently under discussion.

Because massive culling raises issues of animal welfare and ethics, fertility control could play an important and effective role in reduction of feral pig populations. Theoretical and empirical models on population dynamics of wildlife show that fertility control may be effective as much, or even more so, as culling to reduce population size (Bradford and Hobbs 2008). Different traditional methods of fertility control are available for some animal populations (zoo/farm/pets) as well as for humans, but have several limitations. In order to achieve efficacious administration, several repeated doses of contraceptive are needed, making them unpractical for use in wildlife. Contraceptive vaccines could represent a valuable alternative to traditional contraceptives because they provide long-term effect without representing any health hazards (Naz and Saver, 2016). Immunocontraception has been tried successfully in several animal species with promising results (Table 1). Even if 100% success in immunization of feral pigs were not feasible, all the successfully immunized feral pigs would play an indirect role in reducing the resources available to the entire feral pig population in a specific area, including the non-immunized individuals. The aim of this article was to review the progress of immunocontraception use in feral pigs, providing a review of the current status and future perspectives.

Evaluation of population size and dynamics

Feral pig populations may be difficult to monitor for size and dynamics, but an accurate estimate of them is needed if efficacy of a contraceptive vaccination is to be explored. The difficulty in monitoring is due to their preference for habitats that are not easily accessed by human beings, such as forested (Boitani et al., 1995) and mountainous areas (Acevedo et al., 2006). Family groups in which growing piglets are represented may especially seek for shelter in inner forests, whereas adult males may be more prone to wander into the neighboring human settlements (Guo et al., 2017).

Typically, feral pigs live in family-based small groups of 1-6 individuals that may differ in terms of genotype, geography, gender, age and season (Massei et al., 2015; Veličković et al., 2016; Guo et al., 2017). Females live together with their piglets while adult males tend to be solitary for most of the year and form groups only for limited periods in autumn and winter (Fernandez-Llario et al., 1996; Rosell et al., 2004). For instance, infrared camera trap surveys may be utilized to evaluate fluctuations in size of subpopulations over seasons as well as gender- and age distributions (Guo et al., 2017). Changes in environment, climate and genetics may contribute to the differences among populations on different continents. In China, groups seem to be smaller than in Europe, and there seems to be a lower proportion of piglets compared with young adults and adults. However, the sex ratio in China appears similar to that found in Europe (Fernandez-Llario and Mateos-Quesada, 2003; Guo et al., 2017). These differences may at least partly be attributed to the differences in methods used to determine age. In Europe, age estimation has been based on teeth analysis (eruption, wear; Boitani et al., 1995; Merta et al., 2015; Fernandez-Llario and Mateos-Quesada, 2003), whereas the Chinese study utilized body size and striping of the back as indicators of age (Guo et al., 2017). Both invasive and non-invasive methods have been used for evaluation of the size of feral pig populations. Sweitzer et al. (2000) described a mark-sighting approach in certain regions of California to estimating the size of the population. They trapped the pigs and sedated them in order to mark them with color-coded ear tags. The benefit of this method appears to be a high frequency of successful sighting rate by automated cameras and additional information gathered by the procedure, such as teeth examination, lactation and pregnancy status of females, sampling for genetic studies and body condition (Sweitzer et al., 2000). Similar capture protocols were thereafter used to study the contribution of domestic pig breeds to the feral pig genome (Gongora et al., 2003). Parenteral vaccinations could be combined with these methods involving capture of feral pigs, which would considerably increase the value of such a caption. Obvious downsides of these invasive methods, however, include high costs, reduced animal welfare and limitations to the number of animals captured and therefore the representativeness of the samples. In a modern society, the invasive methods of this kind are currently becoming less popular and always have to conform to ethical guidelines.

Non-invasive methods to evaluate the population size and dynamics include camera trap surveys (Guo et al., 2017). This approach involves combination of the latest technology, such as infrared cameras and GPS signaling, in a way that does not involve capture of the animals or other invasive procedures. These kind of direct observations can be combined with those used by hunting clubs. In addition, methods involving simulations based on track counts, utilizing a triangular area of observation or a transect, as in the Formozov-Malyshev-Pereleshin method (Keeping and Pelletier, 2014), may represent a valuable addition for improving overall accuracy of methods used to estimate population size and dynamics.

In conclusion, there is a clear need to develop a reliable and reasonable way of estimating the size of the population in a region of interest. This is the key issue when assessing the efficacy of different types of vaccine - otherwise one will never be able to determine if the vaccine is effective or not. The traditional invasive, capture-recapture methods used for population size evaluation appear complex and not very useful for wild pigs. Instead, the non-invasive methods incorporating the latest technology, direct observations and track counting based modeling appear currently to be the most valuable, especially if used in combination to improve accuracy.

Immunocontraception in feral pigs

Two different types of molecule have been studied as targets for development of contraceptive vaccines in animals, including pigs. These are either the regulators of gamete production, like gonadotropin-releasing hormone, follicle-stimulating hormone and luteinizing hormone, or the regulators of gamete function, such as sperm-specific antigens and zona pellucida proteins. In pigs the two major molecules that have been extensively investigated as principal antigens for immunocontraception are the gonadotropin-releasing hormone (GnRH) and the proteins of the zona pellucida.

GnRH vaccines

GnRH peptides are produced in the hypothalamus and released in a pulsatile manner, leading to stimulation of the pituitary gland and thereby release of gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Millar et al., 2004). Because LH and FSH stimulate ovulation in females and spermatogenesis in males, GnRH is an excellent contraceptive target to be used for both males and females. GnRH is a non-immunogenic peptide and it must be conjugated to a carrier protein to make it immunogenic (Meeusen et al., 2007). There are many GnRH vaccines approved for wild and domestic animals (Miller et al., 2008; Boedeker et al., 2012; Table 1), and one is specifically registered for domestic pigs with the main aim of preventing boar taint in males to be slaughtered (Dunshea et al., 2001). This vaccine could be used with a similar degree of success in feral pigs, if a successful and inexpensive way of administration were found. In domestic pigs, this vaccine consists of two injections to induce a temporary contraceptive and consequently a reduction in boar taint expressing factors (androstenone and skatole) in intact male pigs (Dunshea et al., 2001). According to Killian et al. (2006), injection of different titers of GnRH vaccine to male and female feral pigs stimulated different immune responses: females developed higher titers of specific antibodies with a 2000 µg dose, and males had higher titers with a 1000 µg dose. A single intramuscular injection of a specific gonadotropin-releasing hormone vaccine designed for use in wild animals (GonaCon, National Wildlife Research Center, Fort Collins, Colorado) successfully induced infertility in male (Campbell et al., 2010) and female (Killian et al., 2006) feral pigs. GnRH vaccination decreases the number of estrous cycles in females, which contrasts with PZP vaccination, which increases the number of cycling females (Miller et al., 2000). However, GnRH vaccines do not cause any undesired health effects in males or females (Dunshea et al., 2001; Curtis et al., 2008). Interestingly, vaccinated animals were in better body condition than unvaccinated ones (Gionfriddo et al., 2011), which could be connected with improved feed conversion ratio in immunized pigs (Dunshea et al., 2001).

Zona pellucida vaccines

In mammals, the female gamete is surrounded by a proteic membrane known as the zona pellucida (ZP). ZP glycoproteins are involved in species-specific sperm-egg binding, and protect the oocyte before implantation (Harris et al., 1994). Raising antibodies against these ovum protein receptors for sperm can be used to inhibit fertilization. The use of porcine ZP (PZP) was the initial choice for vaccine development because porcine oocytes are obtained easily from slaughterhouses (Naz and Saver, 2016). This type of vaccine (PZP) has been used at least in horses, elephants, bison, deer and elk (Table 1). Although the amounts of individual ZP components differ among different species, some dimers of the sequence are conserved among mammalian species, thus enabling PZP to be efficiently used in other species (Stetson et al., 2012). In many species, intramuscular injections of raw porcine ZP protein (ZPZ) caused the female to raise antibodies against the sperm receptors on the ovum, effectively inhibiting fertilization (Kirkpatrick and Turner, 1994). Currently there are many PZP vaccines on the market to be used for domestic, farm, zoo and wild animals (Naz and Saver, 2016; Table 1). PZP vaccines have been confirmed to be reversible in mares and deer, if vaccinated once or multiple times with variable time to regain fertility (1– 6 years) (Miller et al., 2000; Kirkpatrick and Turner, 2002). The formulation and dose of PZP varies greatly among studies, ranging from 50 to 600 µg (Naz and Saver, 2016). Infertility and PZP antibody titers are closely correlated, when titers exceed a certain threshold the animals become infertile and when titers revert, the individual regains its fertility (Liu et al., 1989). However, there are few studies on the efficacy of PZP vaccine in feral pigs.

Future perspectives for immunocontraception in feral pigs

The most common contraceptive vaccines available have shown good efficacy in feral pigs, but all need to be administered intramuscularly. This is not an issue in animals that can be readily handled (farms, zoo, natural reserves). In free-roaming wildlife, including feral pigs, however, handling is not feasible. Even remote delivery of the antigen with dart or biobullet is not practical because of the wide spread of feral pig populations, their nocturnal nature and rapid movements. Darting would be similar to a hunting situation, in order to escape human chasing, the feral pig population would move faster in the area, increasing the risk of driving feral pigs into more distant areas. It would be of great advantage if the vaccine could be administered orally. Oral vaccination is scarcely used in animals and humans, with the exception of the oral polio vaccine, mainly because it requires larger quantities of antigen and the immune response is less predictable than with injections (Miller et al., 1998). There are some successful experiences of using an oral vaccination against a pathogen in wildlife in conjunction with a viral disease outbreak. One such positive example is the outbreak of *rabies* in Finland in 1988-1989, in which the disease was found in some domestic species (cat, dog, cow) and wildlife like the raccoon dog population, the red foxes and the badgers. A highly successful oral vaccination campaign involving these wild species was mounted soon after the detection of the outbreak. Finland was declared free from the disease only 1-2 years after the outbreak, due to the successful vaccination campaign (Nyberg et al., 1992). The oral vaccine used was based on Tübingen Fox Baits consisting of fat and fish meal, each containing 1.8 ml live attenuated SAD-B19 rabies virus in a plastic-tinfoil capsule (Nyberg et al., 1992). The baits contained tetracycline as a biomarker. In the first phase of vaccination, the baits were distributed by hunters on an area of 2400 km2 and in a later phase by air distribution over an area of 225 km2. The uptake of the baits were monitored both by observing the baits directly (species recognized by tooth marks in a proportion of cases) and by detection of the tetracycline biomarker in animals brought to autopsy (Nyberg et al., 1992). The main target of oral vaccination in mammals is the mucosal immune system, including the tonsils, and the immune follicles of the small intestine, such as Peyer's patches (Mestecky and McGhee 1989). When targeting the intestinal Peyer’s patches the antigens (proteins) should be protected from stomach digestion, therefore immunization of the pharyngeal area is more feasible (Miller et al., 1999). In the last two decades, understanding of the mechanisms used by viruses and bacteria to colonize the intestinal tract have opened new possibilities for developing successful and safe oral vaccines. Bacteriophage viruses have stimulated interest as possible carriers of specific antigens to be presented to the host to induce adaptive immune responses and humoral and/or cell-mediated immunity (Aghebati-Maleki et al., 2016). Bacteriophages are able to infect and replicate in bacteria but are not pathogenic to animals, including humans. Bacteriophages are stable in the gastrointestinal tract and thus increase their potential use as carriers for oral vaccines (Bazan et al., 2012). The immunostimulatory effects of phage-based vaccines via oral administration were confirmed in various species (Delmastro et al., 1997; Ren et al., 2008). To act as vaccines, phage particles can be re-engineered genetically or modified chemically to carry desirable antigenic domains (Samoylova et al., 2017). A hypothetical immunocontraception oral vaccine for feral pigs would need to be distributed in uncontrolled environments. Therefore, it might be taken up also by non-target species, which renders species-specific preparations preferable (Samoylova et al., 2012). Samoylova et al. (2017) suggested that next generation sequencing (NGS) is a powerful tool that has the potential to accelerate and improve isolation of target-specific phages from phage display libraries. Although bacteriophages are viruses that specifically infect bacterial cells, a range of convincing evidence strongly supports the idea that these prokaryotic viruses can also deliver their encoded genes into mammalian cells.

In conclusion, phage-based preparations have already proved to be immunogenic after oral administration (Delmastro et al., 1997; Zuercher et al., 2000; Ren et al., 2008), indicating a realistic opportunity to develop practical contraceptive vaccines for use in feral pigs. It is evident that in the near future there will be a potential for effective contraceptive vaccines to be used in controlling overpopulation of feral pigs. However, more investigative efforts and resources should be allocated to identify and exploit possible immunocontraception methods based on bacteriophage platforms, in order to develop a species-specific vaccine for feral pigs that is both efficacious and safe to use.

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Table 1. Contraception methods in different species.

Species	**Method of contraception and common route of administration**	**Related references**
Feral pig (*Sus scrofa*)	GnRH-vaccine, intramuscular injection	Massei et al., 2012, Bevins et al., 2014
Cattle (*Bos taurus/Bos indicus*)	GnRH-vaccine, intramuscular injection	Massei et al., 2015
Horse (*Equus caballus*)	GnRH-vaccine, intramuscular injection PZP-vaccine, intramuscular injection	Nuñez et al., 2017, Gray et al., 2010
Dog (*Canis lupus familiaris*)	GnRH agonist implant, subcutaneous administration Chemosterilants, testicular injection GnRH-vaccine, intramuscular injection	Bertschinger et al., 2002, Herbert et al., 2005, Wang M 2002, Massei et al., 2013, Rhodes 2017
Cat (*Felis catus*)	Cabergoline, oral administration by bait GnRH-vaccine, intramuscular injection	Munson et al., 2006, Munson et al., 2001, Bertschinger et al., 2002, Herbert et al., 2005 Robbins et al., 2004, Levy et al., 2005, Levy 2011, Rhodes 2017, Jöchle et al., 1993.
Elephant (*Ioxodonta Africana*)	PZP-vaccine, intramuscular injection	Fayrer-Hosken et al.,1999
Bison (*Bison bison*)	GnRH-vaccine, intramuscular injection PZP-vaccine, intramuscular injection	Duncan et al., 2017, Miller et al., 2004

Deer (*Odocoileus virginianus*)	PZP-vaccine, intramuscular injection	Turner et al., 1992, Turner et al., 1996
Elk (*Cervus elaphus*)	PZP-vaccine, intramuscular injection	Kirkpatrick et al., 1996
Ground squirrel (*Spermophilus beecheyi*)	GnRH-vaccine, intramuscular injection	Yoder et al., 2011