The 1st International Scientific Conference Biotechnology of Farm Animals
Slovak J. Anim. Sci., 46, 2013 (4): 155-159

ISSN 1337-9984

GENETICALLY MODIFIED ANIMALS AS POTENTIAL GENETIC RESOURCES

P. MAJOR, A. KEREKES, G. SKODA, L. HIRIPI, ZS. BŐSZE*

Animal Biotechnology Institute, Gödöllő, Hungary

ABSTRACT

Recent genome programs in livestock animals revolutionized genetic selection. Genomic selection is a form of marker-assisted selection in which genetic markers covering the whole genome are used to estimate genomic breeding values.
Recombinant proteins produced by GMO livestock animals with high pharmaceutical value are already reached the market and these products expected to spread in the forthcoming years. Unfortunately even this approach, namely the production of recombinant proteins by livestock animals is developing more slowly than it potentially could. Pharmaceutical companies may be reluctant to adopt these techniques just because they are new and because they suffer from the GMO negative image but perhaps also because they presently make substantial profit with proteins prepared from cultured cells.
With the advent of novel transgenic technologies the number of valuable GMO large animals as models of human diseases for translational research has been growing exponentially. The emerging novel method, genome editing, which enables the targeted transfer of a favourable allele, leaving unaltered otherwise the genome of the animal and therefore rising less bioethical and regulatory issues might contribute to increase productivity and in parallel sustainability of livestock production in the near future.

Key words: transgenic animal; application; genetic resources

INTRODUCTION

Although population growth in developed nations has reached a plateau, no slowdown is predicted in the developing world until about 2050, when the population of the world is expected to reach 9 billions (United Nations, 2008). To meet the global food demand will require nearly double the current agricultural output, and 70 % of that increased output must come from existing or new technologies (United Nations, 2002).

Recent genome programs in livestock animals revolutionized genetic selection. Genomic selection is a form of marker-assisted selection in which genetic markers covering the whole genome are used to estimate genomic breeding values. Although predicting genetic merit using DNA diagnostics may be less precise than directly testing the performance of every animal, the reduction in generation interval by far offsets it. However even this approach has limitations, since relies on existing genetic variation. If a trait such as disease resistance does not exist in the population it is not possible to select for it. The characterization of novel, economically important allelic forms of genes, or newly described genes in disease resistance could be the outcome of research with genetically modified model organisms where their effect on fitness can be evaluated.

Phenotypic effects of different genes are variable, therefore genetic modification is appropriate to add major effect genes, whereas genetic selection is applied to all genes including the lesser-effect genes.

Transgenic technology has been originally developed in basic research to examine gene functions in model animals, but scientists proposed that this

***Correspondence:** E-mail: bosze@abc.hu
Zsuzsanna Bősze, Animal Biotechnology Institute,
Agricultural Biotechnology Center,
Szentgyörgyi A. st. 4., H-2100 Gödöllő, Hungary
Tel.: +36 (28) 526150 Fax: +36 (28) 526151

Received: August 5, 2013
Accepted: September 5, 2013

technology might have immediate applications for both biomedical and agricultural purposes, when extended to livestock animals.

The applications of transgenic (GMO) livestock technology in biomedicine are in more advanced stage due to greater economical incentive and public acceptance. The main biomedical applications of GMO livestock are (1) biopharming (live bioreactors) for large scale production of pharmaceutically important proteins, (2) xenotransplantation: animal to human transplantation of "humanised" organs and (3) livestock animal models for human diseases.

The main areas of agricultural applications of transgenic technology are (1) increased production efficiency, (2) improved animal welfare and health, (3) improved food safety and quality and (4) reduced environmental footprint.

BIOMEDICAL APPLICATIONS

(1) Biopharming

Recombinant protein expression in milk of GMO animals has been extensively studied in the last twenty years and underwent improvement recently both from methodological point of view and in terms of reaching market. The U.S. FDA issued its first approval for a biological product produced by genetically engineered animals in 2009, for ATryn (human antithrombin III) to treat a rare clotting disorder www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2009/ucm109074.htm.

Patients suffering from hereditary antithrombin deficiency are at high risk of blood clots during invasive medical interventions, such as surgery, and during and after childbirth. ATryn is a therapeutic protein derived from the milk of goats; the current manufacturer is rEVO Biologics - formerly GTC Biotherapeutics, Inc. - (www.gtc-bio.com). The amount of ATryn purified per year from transgenic goats is equivalent to those obtained from 90 000 human blood samplings. Other recombinant proteins produced in bovine, goat or rabbit milk are in the pipeline, at different phases of clinical trials. Ruconest™ (Rhucin® in non-European territories) is a recombinant human C1 inhibitor approved for the treatment of angioedema attacks in patients with hereditary angioedema in all EU countries and produced in rabbit milk by Pharming Group (http://www.pharming.com/).

Transgenic rabbits carrying a 110 kb rabbit genomic fragment encoding the IgG binding Fc receptor (FcRn) were created (Catunda *et al.*, 2012). The FcRn transgenic rabbits showed improved IgG protection and augmented humoral immune response indicating that FcRn overexpression on a large genomic fragment brings significant advantages for the production of polyclonal antibodies (http://www.immunogenes.com).

(2) Xenotransplantation

The gap between the number of patients suffering from complete organ failure and the number of donors is increasing in Western populations and became a life threatening problem for them. The chronic shortage of human organs for transplantation initiated research alternatives to human organs, namely the use of organs from animals as xenografts and stem cell therapy. Pigs are currently thought to be the best candidates for xenotransplantation. The risk of cross-species disease transmission is decreased because of their phylogenetic distance from humans. They are readily available; pig organs are similar in size and physiology to human organs. New infectious agents are less likely since they have been in contact with humans through domestication for many generations. The importance of this research field is underlined by the 4936 publications in PubMed (http://www.ncbi.nlm.nih.gov/pubmed).

Current experiments in xenotransplantation most often use GMO pigs as the donor, and baboons as human models. The most profound barrier to pig-to-primate xenotransplantation is the rejection of the grafted organ by a cascade of immune mechanisms commonly referred to as hyperacute rejection, acute humoral xenograft rejection, immune cell-mediated rejection, and chronic rejection. The protective efficacy of all strategies is strictly dependent on a sufficiently high expression level of the respective factors with the required spatial distribution. Multi-transgenic pigs for clinical xenotransplantation which combine the most important genetic modifications with three, four, or five different genetic modifications were produced recently (Ayares *et al*., 2013). The islets of the multi transgenic pigs were tested in diabetic monkeys and demonstrated prolonged survival, function and complete normalization of blood glucose levels for up to 1 year.

(3) Livestock animal models for human diseases

The domesticated pig has turned into an important disease model, and preclinical testing of novel therapies in human disorders. The cystic fibrosis model pig, created with recombinant adeno-associated virus vector and somatic nuclear transfer to delete the porcine gene developed a disease remarkable similar to human (Rogers *et al*., 2008; Stoltz *et al*., 2013). Using Sleeping Beauty DNA transposition and cloning by somatic cell nuclear transfer, minipigs expressing a human gain-of-function mutation in the proprotein convertase subtilisin/kexin type 9 gene were created. Initial characterization revealed severe hypercholesterolemia, and spontaneous development of progressive atherosclerotic lesions (Al-Mashhadi *et al.*, 2013). Duchenne muscular dystrophy and diabetes porcine models were created in E. Wolf's laboratory with targeted gene mutation and somatic nuclear transfer (Klymiuk *et al*., 2013; Renner

et al., 2013). An autosomal dominant mutation resulting retinitis pigmentosa in humans was modelled in minipigs with somatic nuclear transfer (Ross *et al.*, 2012).

Like pig, the rabbit is more prosperous as an experimental animal model than the rodents in different biomedical aspects. Long-lasting effects of early prenatal development on health and complex diseases (*hypertension, atherosclerosis, diabetes mellitus, hyperlidemia, and obesity*) cannot be adequately mimicked in mice (Duranthon *et al*., 2012). Transgenic rabbits expressing GFP (green fluorescent protein) provide a way to visualisation of fine anatomical structures and cell morphology through in vivo imaging (Katter *et al*., 2012). Beyond that GM rabbit models of human cardiac disorders-cardiac electrophysiology and cardiac hypertrophy- turned out to be extremely useful in pharmacological studies and disease prevention (Senthil *et al*., 2005; Bentzen *et al*., 2011). The first transgenic rabbit model of retinitis pigmentosa (Kondo *et al*., 2009), carries a 100 kb long rabbit rhodopsin BAC clone which was modified to model a dominant negative human mutation. Contrary to rodent models rabbit has large eye and the scientific community has substantial knowledge on its anatomy and ophthalmology (Jones *et al*., 2011), therefore more useful in translational experiments into clinical practice.

AGRICULTURAL APPLICATIONS

(1) Increased production efficiency

There are theoretical limitations to the production capacity of livestock because of the limits of genetic diversity that is available for selection and propagation. It is unlikely that existing genetic variation will continue to generate the rate of gain obtained in the past. It is very likely that genomic selection and genetically-modified animals will be required and that they will may be accepted. After more than 15 years of research and development the transgenic atlantic salmon expressing the chinook salmon growth hormone gene (AquAdvantage salmon developed by AquaBounty Technologies), are already on the way to the table, albeit with considerable opposition from environmental groups (Ledford, 2013). By the end of May 2013, the public comment period officially ended. The FDA is now scheduled to finalize its assessment.

(2) Improved animal welfare and health

A recent publication with the title: New phenotypes for new breeding goals in dairy cattle stated that „New breeding goals should be defined in dairy cattle to face new challenges for sustainable production, to restore functional traits and to address societal demands" (Boichard *et al*., 2012). Among others this publication proposes that global climate change will increase pathogen pressure, raising new questions to be answered.

Transgenic cattle produced in the USA express antibacterial protein lysostaphin in their milk, which dramatically enhanced the resistance of these cows to infection by Stapylococcus aureus, the most common cause of mastitis. This genetic improvement could improve the well-being of millions of dairy cattle and decrease the economical costs of mastitis ($2 billion per year in the USA).

The two main limitations of the agricultural approaches are:

i.; the dissemination of the transgene in herds through artificial insemination is slower than in plants;

ii.; the lack of public acceptance, which may shy away potential producers.

Beyond that, any GMO strategy would need to be more cost effective than vaccination and other disease prevention strategies to compete and to become the preferred disease prevention policy. Currently, there are no treatments for more than 50 % of all livestock diseases. Even for those diseases for which treatment is available some issues limit their effectiveness e.g. virus serotypes against which the vaccine is not protective or the global concerns about the expansively use of antibiotics (Wall *et al*., 2009). Transgenic technology offers novel disease prevention strategies using RNA interference. This strategy is very promising to attack virus-caused diseases (foot and mouth disease, avian influenza, porcine reproductive and respiratory syndrome).

(3) Improved food safety and quality

Current production systems provide safe animal food products with good nutritional qualities but there is room for improvement. Functional foods are increasingly fashionable in the industrialized world. Milk is an important food and therefore introduction of antimicrobial properties into milk could be beneficial for the consumer. GMO goats that are producing the human antibacterial protein lysozyme in their milk were created in the USA (Maga *et al*., 2006). Their milk was consumed by pigs as a human model of the gastrointestinal tract and showed beneficial effect on their intestinal microflora. This GMO goat milk could be left at room temperature for at least two days, which is especially important in developing countries (Wall *et al*., 2009).

Transgenic cattle were created in China, which express human lactoferrin and alfa-lactoglobulin to make it more similar for human milk. Genetically modified goat expressing a converting enzyme in its milk, to decrease long-chain saturated fatty acids content and increase to their monounsaturated forms, was created in California. Consumption of this milk with

higher proportions of monounsaturated fatty acids might have beneficial effect on human cardiovascular health (for review: Fahrenkrug *et al*., 2010). A transgenic rabbit model was created to illustrate the unique ability of transgenic technology to provide novel foods tailored for specific dietary requirements of patients suffering from the genetic disorder phenylketonurea. In this study, a low phenylalanine mutant rabbit κ-casein was expressed at high level in milk and this modified κ-casein could be purified with a simple one step purification step in the Agricultural Biotechnology Centre, Gödöllő, Hungary (Baranyi *et al*., 2007).

(4) Reduced environmental footprint

Livestock transgenesis could contribute to keep agriculture sustainable. The main objects are the following: more effectively utilize both feed and animal resources and reduce pollution. In the intensive pork industry the manure-based environmental pollution is a critical problem. The transgenic pig producing phytase enzyme called Enviropig was developed at the University of Guelph by Canadian scientists (http://www.uoguelph.ca/enviropig/). The pigs are expressing phytase in a saliva specific way, which allows the pigs to digest phytate, the most abundant plant derived source of phosphorus in the pig diet. Phytate passes undigested into manure without this enzyme, increasing the environmental load of pork production. This genetic modification reduces the excretion of undigested phosphor in faces by 30-60 %, which could ameliorate surface water eutrophisation and the environmental footprint of phytase production as food supplement (Golovan *et al.*, 2001). In 2010, Canada has approved limited production of the Enviropigs, in strictly controlled environments for further research. It seemed to be one of the first approval for a meat originated from genetically engineered livestock for human consumption.

The University of Guelph stopped the program and euthanized the pigs, in 2012 after it couldn't find any partner to fund the project. However, the genetic material will be stored at the Canadian Agricultural Genetics Repository Program.

Ecotoxicological studies resulted transgenic fishes which produce special reporter proteins upon environmental stress (Seok *et al*., 2008).

ACKNOWLEDGEMENT

This work was supported by grants by the Hungarian grants OTKA NK 104397 and ANR-NKTH-Regulomix.

REFERENCES

AL-MASHHADI, R. H. – SØRENSEN, C. B. – KRAGH, P. M. – CHRISTOFFERSEN, C. – MORTENSEN, M. B. – TOLBOD, L. P. – THIM, T. – DU, Y. – LI, J. – LIU, Y. – MOLDT, B. – SCHMIDT, M. – VAJTA, G. – LARSEN, T. – PURUP, S. – BOLUND, L. – NIELSEN, L. B. – CALLESEN, H. – FALK, E. – MIKKELSEN, J. G. – BENTZON, J. F. 2013. Familial hypercholesterolemia and atherosclerosis in cloned minipigs created by DNA transposition of a human *PCSK9* gain-of-function mutant. *Sci. Transl. Med*., vol. 5 (166), 2013, p. 166ra1.

AYARES, D. – PHELPS, C. – VAUGHT, T. – BALL, S. – MONAHAN, J. – WALTERS, A. – GIRALDO, A. – BERTERA, S. – VAN DER WINDT, D. - WIJKSTROM, M. – COOPER, D. K. C. – BOTTINO, R. – TRUCCO, M. 2013. Multi-transgenic pigs for xenoislet transplantation. *Xenotrans-plantation*, vol. 20 (1), 2013, p. 46.

BARANYI, M. – HIRIPI, L. – SZABÓ, L. – CATUNDA, A. P. – HARSÁNYI, I. – KOMÁROMY, P. – BOSZE, Z. 2007. Isolation and some effects of functional, low-phenylalanine kappa-casein expressed in the milk of transgenic rabbits. *J. Biotechnol*., vol. 128 (2), 2007, p. 383-392.

BENTZEN, B. H. – BAHRKE, S. – WU, K. – LARSEN, A. P. – ODENING, K. E. – FRANKE, G. – STORM VAŃS GRAVESANDE, K. – BIERMANN, J. – PENG, X. – KOREN, G. – ZEHENDER, M. – BODE, C. – GRUNNET, M. – BRUNNER, M. 2011. Pharmacological activation of Kv11.1 in transgenic long QT-1 rabbits. *J. Cardiovasc. Pharmacol.,* vol. 57 (2), 2011, p. 223-230.

BOICHARD, D. – BROCHARD, M. 2012. New phenotypes for new breeding goals in dairy cattle. *Animal*. vol. 6 (4), 2012, p. 544-550.

CATUNDA LEMOS, A. P. – CERVENAK, J. – BENDER, B. - HOFFMANN, O. I. – BARANYI, M. – KEREKES, A. – FARKAS, A. – BOSZE, Z. – HIRIPI, L. – KACSKOVICS, I. 2012. Characterization of the rabbit neonatal Fc receptor (FcRn) and analyzing the immunophenotype of the transgenic rabbits that overexpresses FcRn. *PLoS One*, vol. 7 (1), 2012, e28869.

DURANTHON, V. – BEAUJEAN, N. – BRUNNER, M. – ODENING, K. E. – SANTOS, A. N. – KACSKOVICS, I. – HIRIPI, L. – WEINSTEIN, E. J. – BOSZE, Z. 2012. On the emerging role of rabbit as human disease model and the instrumental role of novel transgenic tools. *Transgenic Re*s., vol. 21 (4), 2012, p. 699-713.

FAHRENKRUG, S. C. – BLAKE, A. – CARLSON, D. F. – DORAN, T. – VAN EENENNAAM, A. – FABER, D. – GALLI, C. – GAO, Q. – HACKETT, P. B. – LI, N. – MAGA, E. A. – MUIR, W. M. – MURRAY, J. D. – SHI, D. – STOTISH, R. – SULLIVAN, E.

– TAYLOR, J. F. – WALTON, M. – WHEELER, M. – WHITELAW, B. – GLENN, B. P. 2010. Precision genetics for complex objectives in animal agriculture. *J. Anim. Sci*., vol. 88 (7), 2010, p. 2530-2539.

GOLOVAN, S. P. – MEIDINGER, R. G. – AJAKAIYE, A. – COTTRILL, M. – WIEDERKEHR, M. Z. – BARNEY, D. J. – PLANTE, C. – POLLARD, J. W. – FAN, M. Z. – HAYES, M. A. – LAURSEN, J. – HJORTH, J. P. – HACKER, R. R. – PHILLIPS, J. P. – FORSBERG, C. W. Pigs expressing salivary phytase produce low-phosphorus manure. *Nat. Biotechnol*., vol. 19 (8), 2001, p. 741-745.

JONES, B. W. – KONDO, M. – TERASAKI, H. – WATT, C. B. – RAPP, K. – ANDERSON, J. – LIN, Y. – SHAW, M. V. – YANG, J. H. – MARC, R. E. 2011. Retinal remodeling in the Tg P347L rabbit, a large-eye model of retinal degeneration. *J. Comp. Neurol*., vol. 519 (14), 2011, p. 2713-2733.

KATTER, K. – GEURTS, A. M. – HOFFMANN, O. – MÁTÉS, L. – LANDA, V. – HIRIPI, L. – MORENO, C. – LAZAR, J. – BASHIR, S. – ZIDEK, V. – POPOVA, E. – JERCHOW, B. – BECKER, K. – DEVARAJ, A. – WALTER, I. – GRZYBOWKSI, M. – CORBETT, M. – FILHO, A. R. – HODGES, M. R. – BADER, M. – IVICS, Z. – JACOB, H. J. – PRAVENEC, M. – BOSZE, Z. – RÜLICKE, T. – IZSVÁK, Z. 2013. Transposon-mediated transgenesis, transgenic rescue, and tissue-specific gene expression in rodents and rabbits. *FASEB J.: official publ. of the Fed. of American Soc. for Experimental Biol.,* vol. 27 (3), 2013, p. 930-941.

KLYMIUK, N. – BLUTKE, A. – GRAF, A. – KRAUSE, S. – BURKHARDT, K. – WUENSCH, A. – KREBS, S. – KESSLER, B. – ZAKHARTCHENKO, V. – KUROME, M. – KEMTER, E. – NAGASHIMA, H. – SCHOSER, B. – HERBACH, N. – BLUM, H. – WANKE, R. – AARTSMARUS, A. – THIRION, C. – LOCHMÜLLER, H. – WALTER, M. C. – WOLF, E. 2013. Dystrophin-deficient pigs provide new insights into the hierarchy of physiological derangements of dystrophic muscle. *Hum. Mol. Genet*., vol. 22 (21), 2013, p. 4368-82.

KONDO, M. – SAKAI, T. – KOMEIMA, K. – KURIMOTO, Y. – UENO, S. – NISHIZAWA, Y. – USUKURA, J. – FUJIKADO, T. – TANO, Y. – TERASAKI, H. 2009. Generation of a transgenic rabbit model of retinal degeneration. Invest *Ophthalmol. Vis. Sci*., vol. 50 (3), 2009, p. 1371-7.

LEDFORD, H. 2013. Transgenic salmon nears approval. *Nature*, 497, 2013, p. 17-18

MAGA, E. A. – SHOEMAKER, C. F. – ROWE, J. D. – BONDURANT, R. H. – ANDERSON, G. B. – MURRAY, J. D. 2006. Production and processing of milk from transgenic goats expressing human lysozyme in the mammary gland *J. Dairy Sci.*, vol. 89 (2), 2006, p. 518-24.

RENNER, S. – BRAUN-REICHHART, C. – BLUTKE, A. – HERBACH, N. – EMRICH, D . – STRECKEL, E. – WÜNSCH, A. – KESSLER, B. – KUROME, M. – BÄHR, A. – KLYMIUK, N. – KREBS, S. – PUK, O. – NAGASHIMA, H. – GRAW, J. – BLUM, H. – WANKE, R. – WOLF, E. 2013. Permanent neonatal diabetes in INS(C94Y) transgenic pigs. *Diabetes*, vol. 62 (5), 2013, p. 1505-1511.

ROGERS, C. S. – STOLTZ, D. A . – MEYERHOLZ, D. K. – OSTEDGAARD, L. S. – ROKHLINA, T. – TAFT, P. J. – ROGAN, M. P. – PEZZULO, A. A. – KARP, P. H. – ITANI, O. A. – KABEL, A. C. – WOHLFORD-LENANE, C. L. – DAVIS, G. J. – HANFLAND, R. A. – SMITH, T. L. – SAMUEL, M. – WAX, D. – MURPHY, C. N. – RIEKE, A. – WHITWORTH, K. – UC, A. – STARNER, T. D. – BROGDEN, K. A. – SHILYANSKY, J. – MCCRAY, P. B JR. – ZABNER, J. – PRATHER, R. S. – WELSH, M. J. 2008. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. *Science*, vol. 321 (5897), 2008, p. 1837-41.

ROSS, J. W. – FERNANDEZ DE CASTRO, J. P. – ZHAO, J. – SAMUEL, M. – WALTERS, E. – RIOS, C. – BRAY-WARD, P. – JONES, B. W. – MARC, R. E. – WANG, W. – ZHOU, L. – NOEL, J. M. – MCCALL, M. A. – DEMARCO, P. J. – PRATHER , R. S. – KAPLAN, H. J. 2012. Generation of an inbred miniature pig model of retinitis pigmentosa. Invest *Ophthalmol. Vis. Sci*., vol. 53 (1), 2012, p. 501-507.

SENTHIL, V. – CHEN, S. N. – TSYBOULEVA, N. – HALDER, T. – NAGUEH, S. F. – WILLERSON, J. T. – ROBERTS, R. – MARIAN, A. J. 2005. Prevention of cardiac hypertrophy by atorvastatin in a transgenic rabbit model of human hypertrophic cardiomyopathy. *Circ. Res*., vol. 97 (3), 2005, p. 285-292.

SEOK, S. H. – BAEK, M. W. – LEE, H. Y. – KIM, D. J. – NA, Y. R. – NOH, K. J. – PARK, S.H. – LEE, H. K. – LEE, B. H. – PARK, J. H. 2008. In vivo alternative testing with zebrafish in ecotoxicology. *J. Vet. Sci*., vol. 9 (4), 2008, p. 351-357.

STOLTZ, D. A. – ROKHLINA, T. – ERNST, S. E. – PEZZULO, A. A. – OSTEDGAARD, L. S. – KARP, P. H. – SAMUEL, M. S. – REZNIKOV, L. R. – RECTOR, M. V. – GANSEMER, N. D. – BOUZEK, D. C. – ALAIWA, M. H. – HOEGGER, M. J. – LUDWIG, P. S. – TAFT, P. J. – WALLEN, T. J. – WOHLFORD-LENANE, C. – MCMENIMEN, J. D. – CHEN, J. H. – BOGAN, K. L. – ADAM, R. J. – HORNICK, E. E. – NELSON, G. A. – HOFFMAN, E. A. – CHANG, E. H. – ZABNER, J. – MCCRAY, P. B JR. – PRATHER, R. S. – MEYERHOLZ, D. K. – WELSH, M. J. 2013. Intestinal CFTR expression alleviates meconium ileus in cystic fibrosis pigs. *J. Clin. Invest.*, vol. 123 (6), 2013, p. 2685-2693.

WALL, R. – LAIBLE, G. – MAGA, E. – MURRAY, J. – SEIDEL, G. – WHITELAW, B. 2009. Animal Productivity and Genetic Diversity: Cloned and Transgenic Animals. *CAST,* Issue paper, 43, 2009, p. 1-16.