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Journal of Veterinary Medicine and Research

Research Article

# Evaluation of Risks from Environmental Contact with Transgenic Livestock

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**Submitted:** 19 May 2020

**Accepted:** 26 May 2020

**Published:** 28 May 2020

**ISSN:** 2379-948X

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OPEN ACCESS

**Keywords**

- Transgenic swine
- Risk assessment
- α-lactalbumin
- Horizontal gene transmission

**Abstract**

Assessment of the risks associated with exposing non-transgenic animals to transgenic animals is important to the future contributions of transgenic livestock to livestock production and society. Evaluation of the potential for the transfer of a transgene (Tg) from livestock to a non-transgenic animal during parturition, mating, gestation, or lactation is the initial step in a risk assessment. We previously developed and characterized transgenic swine containing a mammary-specific Tg, bovine α-lactalbumin, (Bα-LA) that results in increased milk production in sows. In this study, we wanted to determine whether Bα-LA is expressed in tissues of transgenic swine other than the lactating mammary gland and if the Tg DNA crosses into non-transgenic swine under various physiological and physical conditions. The specific aims addressed in this study were to determine (1) whether the bovine α-lactalbumin protein can be synthesized in any other tissues than the mammary gland of a transgenic sow; (2) whether the Tg can be transferred directly by physical association or contact; (3) whether the Tg can be transferred directly via mating; and (4) whether the Tg can be transferred directly during gestation, parturition, or lactation.

## ABBREVIATIONS

Bα-LA: bovine α-lactalbumin; Bα-LA Tg: bovine α-lactalbumin transgene; C: Control Pig; $CO_2$: Carbon dioxide; DNA: Deoxyribonucleic acid; $H_2O$: Water; Tg: Transgene; PCR: Polymerase Chain Reaction; RT-PCR: Reverse Transcriptase-Polymerase Chain Reaction; T: Transgenic Pig

## INTRODUCTION

In the past two-plus decades, the increasing use of transgenic animals as models in biomedical sciences has raised questions regarding the risk of the transgene transferring from a transgenic animal to a non-transgenic animal. Furthermore, only one transgenic animal, the AquaBounty™ Atlantic Salmon, has been approved for production agriculture (https://aquabounty.com/fda-approval-of-ge-salmon-paves-way-for-sustainable-food-innovations/). The assessment of their risk is an important requirement for the future use of transgenic livestock. In the present study, the term "transgenic" will be defined as "one whose genetic make-up has been modified by the addition or deletion of a specific DNA sequence" [1]. Transgenic technology provides a method to rapidly introduce new genes into plants and animals [2] and has applications to improve production traits, enhance animal health, and develop biomedical models.

Transgenic swine are a critical part of the development of models in research using pigs for biomedical sciences. With the complete sequence of the pig genome now available, we now have the ability to reliably produce transgenic swine. Owing to the overwhelming physiological similarities between pigs and humans, the pig provides a relevant animal model for biomedical sciences [3]. In addition to bioengineering, imaging, and behavioral studies, transgenic methods can be used to enhance productivity traits in swine.

The technology involved in the production of transgenic livestock holds great promise for agriculture and biomedical sciences but also has potential risks. The public's perception of biotechnology tends to be accepting when it is involved in the development of new pharmaceuticals. However, the public is less accepting when dealing with production traits or animals as a food source. It is clear that to realize the long-term benefits of transgenic technology to society, the potential risks of transgenic animals on the environment, producers, consumers, and the animals themselves must be carefully evaluated [4-11].

We have previously developed transgenic pigs that have significantly increased milk production, resulting in increased weaning weights in piglets suckling these transgenic sows [12,13]. The transgenic pigs expressed a mammary-specific transgene,

bovine α-lactalbumin (Bα-LA). α-Lactalbumin is a component of the lactose synthase complex expressed in the mammary gland during lactation. The lactose synthase complex is composed of the constitutively expressed enzyme, β1, 4-galactosyltransferase, which is modified by the interaction with α-lactalbumin. Lactose synthase results in the synthesis of lactose. The activity of the lactose synthase complex may be rate-limiting to the production of lactose and, therefore to milk volume [14]. As the primary osmole in milk, production of lactose in the Golgi apparatus and secretory vesicles draws water into the mammary cell, maintaining the osmotic balance between tissue fluid and milk [15]. The transgenic pigs that express Bα-LA during lactation have higher milk production and rates of gain of their litter than non-transgenic sows [13,16]. Since milk production by the sow is the primary limiting factor to piglet growth [17], lines of transgenic swine, such as the Bα-LA swine, could have a significant impact on piglet growth and health.

Currently, these transgenic swine must be housed separated from the rest of the production herd to deter any form of inadvertent gene transfer. This research aims to determine if bovine alpha-lactalbumin (Bα-LA) is expressed in tissues other than the mammary gland during lactation of transgenic sows and whether the transgene DNA crosses over into non-transgenic pigs under various physiological and physical conditions. These conditions include housing, mating, gestation, lactation, and suckling.

To examine the risk or hazard of transmission, transgenic pigs were studied in a setting where the pigs are born, raised, and maintained. It is important to understand all possible modes of DNA transmission, especially in production settings to get a handle on the possible risks to the environment associated with the use of genetic engineering technologies in domestic farm livestock. The overall hypotheses of these experiments are that the bovine alpha-lactalbumin transgene is not horizontally transmitted from transgenic to non-transgenic swine.

## MATERIALS AND METHODS

### Animals and Tissue Collection

Experimental protocols for this study were approved by the University of Illinois Institutional Animal Care and Use Committee (protocol #07190). In all experiments, non-transgenic pigs (control = C) and pigs heterozygous for the Bα-LA transgene (transgenic = T) were used. The T pigs were produced as described previously [12]. All swine, above 25 kg, were euthanized by restraining them with a limp rope snare and injecting sodium pentobarbital via a peripheral ear vein (65 mg/ml; equivalent to a dosage of 20-25 mg/kg). Pigs less than 25 kg were euthanized by inhalation of $CO_2$. The carcasses of the euthanized swine were disposed of by incineration.

Tissue samples were removed using aseptic techniques. Tissues were cut into ~0.5 mm cubes and immediately frozen and stored in liquid nitrogen until processing. The DNA was extracted from tissues using the method described by Hogan et al. [18]. DNA was amplified by polymerase chain reaction (PCR) using nested primers according to the method described by Monaco et al. [19]. Total cellular RNA was isolated from the tissues as previously described by Monaco et al. [19], and PCR was performed to identify the transgene was performed using methods of Bleck and Bremel [20].

### Screening for the Expression of the Bovine α-lactalbumin Tg

Total RNA was isolated from the tissues and subjected to reverse transcriptase-polymerase chain reaction using random oligo d (T) primers. Concentrations of cDNA were determined by spectrophotometry at 260 and 280 nm. The resulting cDNA samples were subjected to RT-PCR using Taqman Mastermix Reagent Kit (Applied Biosystems, Foster City, CA). Primer sets were designed to detect a 71 base pair DNA sequence within the 3 un-translated region of the bovine transgene transcript and a 78 base pair DNA sequence within the 3′ un-translated region of the endogenous porcine α-LA gene transcript as a control for the reaction. The forward and reverse primers that were used to amplify the bovine α-LA sequence are 5′-GACATGTAAG-GACTAATCTCCAGGG-3′ and 5′AGGGACATCGAGCAA-GGGT-3′, respectively. The forward and reverse primers were to be used to amplify the porcine α-LA sequences are 5′-GTAGTGATTGTTATC-CGGACACTATTCT-3′ and 5′-GGGCACTGAGCA-AAGGTTAAAA-3′, respectively. Regions of the cDNA amplified by these primer sets have at least 4 to 8 base pair mismatches in the cDNA sequences of the bovine transgene and the porcine α-LA gene transcripts to allow for sequence discrimination. A 20 to 25 bp sequence located between the primer sets was used to design fluorescence reporter probes specific for the bovine transgene and the porcine α-LA transcripts. Each probe contains a fluorescent dye conjugated to the 5′ end of the probe sequence and a quencher dye to suppress probe fluorescence conjugated to the 3′ end of the probe. The sequences of the bovine and porcine α-LA probes are 5′ -6FAM-ATGAATGGCGCTCTGGACTT-TAMRA-3′ and 5′ -VIC AGA-GATGCGTGACTGGTGCAC-TGGA-TAMRA-3′, respectively. The 18 s primers and probe sets were run with all samples to normalize the data. The forward and reverse primers that were used to amplify 18s sequence are 5′ -GATCCATTGGAGGGCAAGTCT-3 and 5′ -AACTGCAGCAACTTTAATATACGCTATT-3′, respectively. The 18s probe sequence is 5′ -6FAM-TGCCAGCAGCCGCGGTAATTC-TAMRA3′ (Figure 1).

### Screening for the Bovine α-lactalbumin Tg

DNA from tissue samples or biopsies was extracted [18]. Nested polymerase chain reaction (PCR) was performed using 10 µl 10x PCR reaction buffer (500 mMKCl, 100 mM Tris-HCl (pH=8.8), 15 mM$MgCl_2$, 1% Triton X-100), 200 mM each dNTP, 1.0 µM each primer (Primers 1 and 2 spanned a portion of the **α**-lactalbumin promoter and coding sequence (5’-AAAAAGGTTGGGTCACTCTT-3’ 1160/1630 Forward and 5’-ATTGCTTCACTTGTATTACCC-3’ 1160/1630 Reverse) and the nested primers 3 and 4 (5’-ACTCTGAGGCTGTCTACAAG-3’ 1198/1473 Forward and 5’-CTTGGTTCCTTGTTGAGTGG-3’ 1198/1473 REVERSE) amplified a target sequence inside of the original primer pair), 1 unit Taq DNA polymerase and 1 µg genomic DNA). Volumes were adjusted to 100 µl with double distilled filter-sterilized water, and reaction was overlaid with light mineral oil. Samples were subjected to two rounds of 30 cycles (94°C 2 min., 50°C 1.5 min., 72°C 1.5 min.). The first round used primers 1 and 2, and the second round used the nested primer pair 2 and 4. This gave us the sensitivity to detect a single copy of the target Tg DNA. Products were separated in a 1% agarose gel and stained with ethidium bromide. DNA containing the Tg produced a 700 bp band corresponding to a portion of

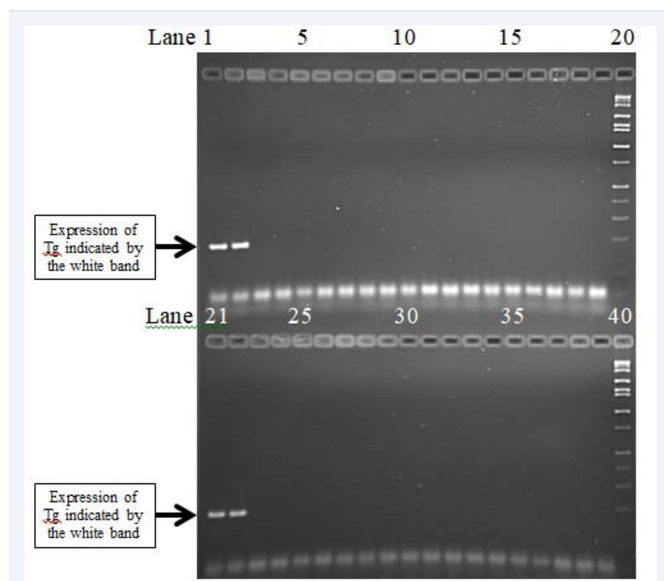


**Figure 1** Example RT-PCR gel to show the presence or absence of the Tg expression in other tissues than the lactating mammary gland. (Lane 1. Day 12 of lactation control, 2. Day 7 of lactation control, 3. H2O control, 4-13. Transgenic tissue samples, 14-16. Non-transgenic tissue samples, 17-19. Transgenic tissue samples, 20. 10 kb Ladder, 21. Day 15 of lactation control, 22. Day 7 of lactation control, 23. H2O control, 24-26. Non-transgenic tissue samples, 27-30. Transgenic tissue samples, 31-34. Non-transgenic tissue samples, 35-39. Transgenic tissue samples, 40. 10kb Ladder).
Tg=transgene

the bovine α-lactalbumin 5' flanking region from the first round amplification. The second round amplification produced a 276 bp band corresponding to the nested DNA sequence (Figure 2). Nested PCR has been shown to amplify single copies of rare DNA sequences [21-23]. One of these groups compared nested PCR and real-time PCR and showed real-time PCR improved the levels of detection of the target DNA sequence, but not to a significant degree [23]. Due to the cost of real-time PCR and the number of samples requiring analysis, we chose to use nested PCR for these studies.

## Experimental Design

**Experiment 1:** The objective of experiment 1 was to determine whether Tg was expressed in any other tissue than the lactating mammary gland. There have been reports of the Tg showing low-level expression in the skin (sebaceous gland) [24]. For this experiment, 11 T pigs (2 males and 9 females) and 11 C pigs (3 males and 8 females) were used. Pigs were raised to 180, 220, 250 d of age, or 112 d post-breeding and then sacrificed for tissue collection. Tissues collected from each pig included blood, brain, jejunum, kidney, liver, lung, mammary gland, muscle, ovary, sublingual salivary gland, skin, and spleen. PCR was used to detect the expression of Tg.

**Experiment 2:** The objective of experiment 2 was to determine whether the co-habitation of T and C pigs would result in the transfer of the Tg to the C pigs. For this experiment 41 T pigs (24 males and 17 females) and 54 C pigs (29 males and 25 females). The number and genotype of the pigs used in each treatment group of this experiment are shown in Table 1. These pigs were raised in pens of four (2 T pigs and 2 C pigs) from weaning (approx. 21 d of age) until they reached either 180, 220 or 250 d of age. At each time, 2 T males, 2 T females, 2 C males, and 2 C females were euthanized. Tissues taken from each pig included the blood, brain, jejunum, kidney, liver, lung, mammary gland, muscle, skin, ovary, sublingual salivary gland, and spleen. The presence of the Tg was determined by nested PCR that was designed to detect a single copy of the transgene. The total number of samples analyzed in experiment 2 is shown in Table 4.

**Experiment 3:** The objective of experiment 3 was to determine if there was a transfer of Tg from T pigs to C pigs as a result of mating. Tissues were collected from 4 T females, 34 C females, and 12 C males. Pigs were raised in routine production settings until they reached breeding age (~7 months), at which time the C females were mated to one of 10 T boars. Females then were sacrificed at 2, 7, 90, or 112 d post-mating (10, 10, 10 and 4 females, respectively) and tissue samples were obtained from brain, cervix, jejunum, kidney, liver, lung, mammary gland, muscle, ovary, oviduct, sublingual salivary gland, skin, spleen, uterus, and vagina. The C males were allowed to naturally mate a T female, and then the C males were sacrificed at 7 days post-mating. Samples of the blood, bulbourethral gland, epididymis, kidney, liver, lung, muscle, penis, skin, spleen, testis, and urethra were collected from the C males. Detection of Tg presence was determined by nested PCR.

**Experiment 4:** The objective of experiment 4 was to evaluate the potential Tg transfer from T pigs to C pigs during parturition, gestation, or lactation. Experiment 4 was divided into three sub-experiments. In sub-experiment 4a, there were 2 groups of pigs, Group 1 (newborn non-suckle) had 4 C sows that were bred to a C boar, and 4 T sows were bred to a C boar. These sows were gestated in a routine production setting and allowed to farrow. The piglets were removed immediately after parturition (13 males and 15 females from C sows and 15 males and 15 females from T sows (Table 2). The second group of pigs (Group 2, 112

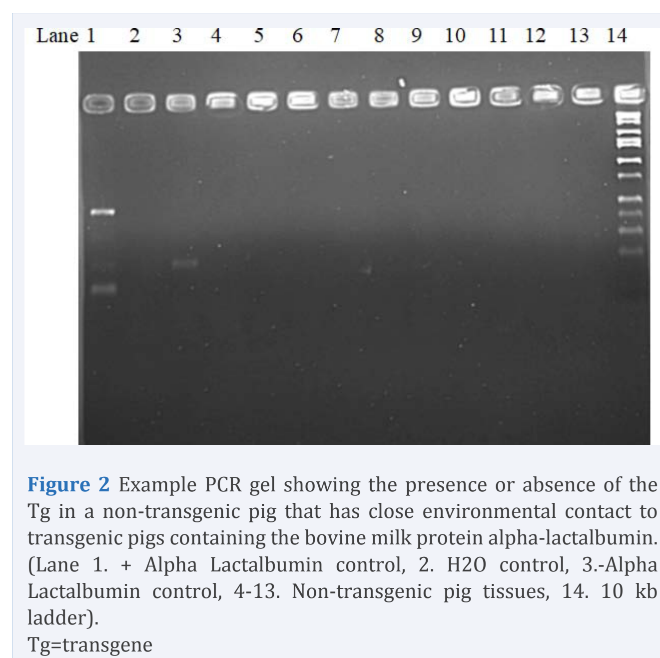


**Figure 2** Example PCR gel showing the presence or absence of the Tg in a non-transgenic pig that has close environmental contact to transgenic pigs containing the bovine milk protein alpha-lactalbumin. (Lane 1. + Alpha Lactalbumin control, 2. H2O control, 3.-Alpha Lactalbumin control, 4-13. Non-transgenic pig tissues, 14. 10 kb ladder).
Tg=transgene

**Table 1:** The number of pigs used for cohabitation study (Experiment 2).

| | | | | Non-Transgenic | | Transgenic[1] | |
|---|---|---|---|---|---|---|---|
| **Treatment** | **# of Pigs[2]** | **# Non-transgenic Pigs[3]** | **# Transgenic Pigs[4]** | **Males** | **Females** | **Males** | **Females** |
| **180 d cohabitation** | 28 | 16 | 12 | 8 | 8 | 5 | 7 |
| **220 d cohabitation** | 27 | 16 | 11 | 8 | 8 | 6 | 5 |
| **250 d cohabitation** | 40 | 22 | 18 | 13 | 9 | 13 | 5 |
| **Total** | **95** | **54** | **41** | **29** | **25** | **24** | **17** |

[1]DNA was isolated from ear biopsies to determine that the Tg was present at the birth of these pigs.
[2]The total number of pigs that were T and non-T combined used in experiment 2.
[3]The number of pigs that were non-T used in experiment 2.
[4]The number of pigs that were T used in experiment 2.
Abbreviations: T=Transgenic pig, C=Control (non-transgenic) pig, Tg=transgene, d=day

**Table 2:** The number of pigs used for parturition and gestation study (Experiment 4a).

| | | | | Non-Transgenic | | Transgenic[1] | |
|---|---|---|---|---|---|---|---|
| **Group** | **# of Pigs[2]** | **# Non-Transgenic Pigs[3]** | **# Transgenic Pigs[4]** | **Males** | **Females** | **Males** | **Females** |
| **1-Newborn/Non-suckle[5]** | 58 | 28 | 30 | 13 | 15 | 15 | 15 |
| **2-112 d Fetuses[6]** | 56 | 25 | 31 | 15 | 10 | 17 | 14 |
| **Total** | **114** | **53** | **61** | **28** | **25** | **32** | **29** |

[1]DNA was isolated from ear biopsies to determine that the Tg was present at the birth of these pigs.
[2]The total number of pigs that were T and non-T used in sub-experiment 4a.
[3]The number of pigs that were non-T used in sub-experiment 4a.
[4]The number of pigs that were T used in sub-experiment 4a.
[5]Piglets that were allowed to farrow normally, but were not allowed to suckle their birth dam.
[6]Fetuses collected at 112 d post-breeding.
T=Transgenic pig, C=Control (non-transgenic) pig, Tg=transgene, d=day

**Table 3:** Presence or absence of Tg expression in tissue samples other than the mammary gland of non-T and T swine analyzed by reverse transcriptase PCR (Experiment 1).

| | | | | Non-Transgenic | | Transgenic[1] | |
|---|---|---|---|---|---|---|---|
| **Treatment** | **# of tissue samples[2]** | **# Non-Transgenic tissue samples[3]** | **# Transgenic tissue samples[4]** | **Present[5]** | **Absent[6]** | **Present[5]** | **Absent[6]** |
| **180 d** | 23 | 11 | 12 | 0 | 11 | 1 | 11 |
| **220 d** | 20 | 9 | 11 | 0 | 9 | 0 | 11 |
| **250 d** | 29 | 12 | 17 | 0 | 12 | 0 | 17 |
| **112 d post-breeding** | 40 | 29 | 11 | 0 | 29 | 0 | 11 |
| **Total** | **112** | **61** | **51** | **0** | **61** | **1** | **50** |

[1]DNA was isolated from ear biopsies to determine that the Tg was present at the birth of these pigs.
[2]The total number of pigs that were T and non-T combined used in experiment 1.
[3]The number of pigs that were non-T used in experiment 1.
[4]The number of pigs that were T used in experiment 1.
[5]The number of tissue samples that had the presence of Tg expression.
[6]The number of tissue samples that had the absence of the Tg expression.
Differential Tg expression between transgenic groups P-value < 0.2465 at 180 d, and P-value >=1 at 220 d, 250 d, and 112 d.
T=Transgenic pig, C=Control (non-transgenic) pig, Tg=transgene, d=day

d fetuses) also had 4 C sows were bred to a C boar and 4 T sows were bred to a C boar. The Group 2 sows gestated in a standard production setting for 112 d of gestation at which point they were euthanized and the fetuses collected (15 males and 10 females from C sows and 17 males and 14 females from T sows (Table 2).

For sub-experiment 4b, newborn C piglets were removed from their birth dam before they suckled and immediately cross-fostered to a lactating T sow or a lactating C sow. The methods used for cross-fostering are described in Figure 3. Piglets were allowed to suckle for 24 h (5 suckling T sow, 5 suckling C sow) or 72 h (6 suckling T sow, 5 suckling C sow) before sacrifice.

In sub-experiment 4c, C piglets were allowed to suckle their birth dam until day 3 and then fostered to a lactating T sow or a lactating C sow (Figure 3). After cross-fostering, the piglets were

allowed to suckle for 72 h (5 suckling T sow, 3 suckling C sow) or 168 h (4 suckling T sow, 4 suckling C sow) before sacrifice. Samples collected from all piglets included blood, jejunum, liver, lung, and muscle. The presence of the Tg was determined by nested PCR in all the studies performed in experiment 4.

### Power and Statistical Analysis

Power analysis indicated that a sample size of 4 and 10 samples per transgenic group (T vs. C) afforded a 88% and 99% statistical power respectively for a positive Tg test with 5% probability and a type I error rate of 5% [25]. A generalized linear model was used to assess the statistical association between Tg expression and transgenic group. The logistic model included the factor of the transgenic group and was implemented using PROC GLIMMIX [26].

## RESULTS

### Experiment 1

A total of 112 tissue samples were analyzed to determine if the Tg is expressed in tissues other than the mammary gland of a T lactating sow. All tissue samples except the salivary gland of one T animal were negative for the expression of the transgene (Table 3).

### Experiment 2

A total of 365 tissue samples were tested from the C pigs that had a direct association or physical contact with T pigs. The tissue samples collected from C pigs were negative for the presence of the Bα-LA transgene (Table 4). Conversely, all samples from T pigs (n=206) were positive for the Bα-LA transgene.

### Experiment 3

A total of 343 tissue samples were analyzed from C sows mated to T boars or from C boars mated to T sows. All C animal tissue samples tested after mating were negative for the presence of the Bα-LA transgene (Table 5).

### Experiment 4

To assess whether the Tg could be transferred through the birthing process (experiment 4a, Group 1), 243 tissue samples were collected from newborn piglets before suckling the dam (Table 6). All tissue samples derived from mating C females to C males were all negative for the presence of the Bα-LA transgene. Nested PCR analysis of the samples taken from piglets derived from mating T females to C males showed that 79 piglets were positive, and 52 piglets were negative for the presence of the Bα-LA transgene. This result is consistent with the T females being heterozygous for the Tg. Furthermore, the absence of the Tg in the negative control piglets indicates that the Tg does not contaminate the piglets during the parturition process.

To determine whether the Tg could be transferred to C fetuses during gestation (experiment 4a, Group 2), 285 tissue samples were analyzed for the Tg (Table 6). There were 127 tissue samples collected from the 112 d fetuses derived from mating C females to C males. All the tissues collected from the C by C matings were negative for the Tg. Analysis of the tissue samples derived from 112-day fetuses resulting from mating of a T female to a C male revealed 99 piglets with the presence of the Tg and 59 piglets negative for the presence of the Tg. Again, this is consistent with the T females being heterozygous for the Tg. The only exception was the outer placental membrane, a tissue derived from the maternal reproductive tract, which was positive

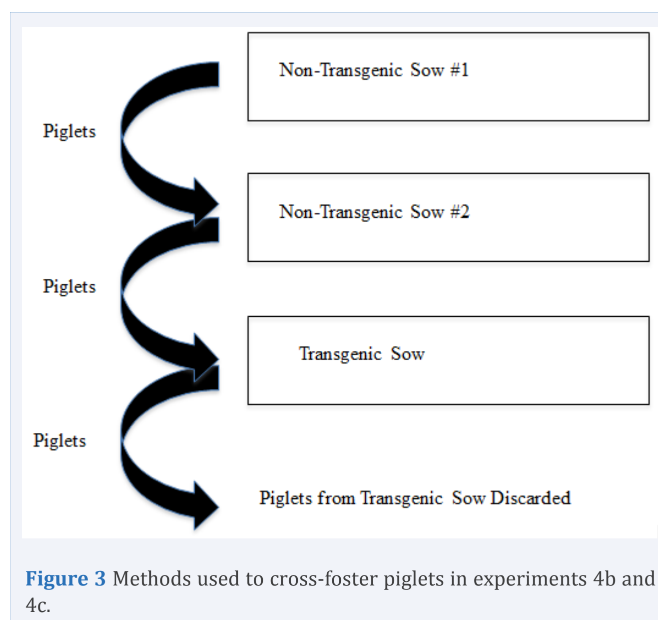


**Figure 3** Methods used to cross-foster piglets in experiments 4b and 4c.

**Table 4.** The number of tissue samples analyzed for cohabitation (Experiment 2).

| | | | | Non-Transgenic | | Transgenic[1] | |
|---|---|---|---|---|---|---|---|
| **Treatment (days)** | **# of tissue samples[2]** | **# Non-Transgenic tissue samples[3]** | **# Transgenic tissue samples[4]** | **Present[5]** | **Absent[6]** | **Present[5]** | **Absent[6]** |
| **180d cohabitation** | 282 | 169 | 113 | 0 | 169 | 113 | 0 |
| **220d cohabitation** | 127 | 80 | 47 | 0 | 80 | 47 | 0 |
| **250d cohabitation** | 162 | 116 | 46 | 0 | 116 | 46 | 0 |
| **Total** | **571** | **365** | **206** | **0** | **365** | **206** | **0** |

[1]DNA was isolated from ear biopsies to determine that the Tg was present at the birth of these pigs.
[2]The total number of pigs that were T and non-T combined used in experiment 2.
[3]The number of pigs that were non-T used in experiment 2.
[4]The number of pigs that were T used in experiment 2.
[5]The number of tissue samples that had the presence of Tg.
[6]The number of tissue samples that had the absence of the Tg.
Differential Tg expression between transgenic groups at 180 d, 220 d, and 250 d P-value < 0.0001
T=Transgenic pig, C=Control (non-transgenic) pig, Tg=transgene, d=day

for all fetuses from the T sows (Table 7).

To assess whether the Tg can be transferred through ingestion of colostrum or milk, tissue samples were collected from C piglets that were cross-fostered to T sows. Samples were collected at either at 0 d (before consuming the birth sow's colostrum; n=87 samples) or at 3 d post-partum (after consuming the birth sow's colostrum (n=70 samples). All tissue samples were negative for the Tg (Table 8).

## DISCUSSION

The present study demonstrates that the Bα-LA Tg 1) is not expressed in tissues other than exocrine glands of transgenic

**Table 5.** The number of tissue samples analyzed for Tg transmission to the C sire or dam after mating (Experiment 3).

| Treatment | # of tissue samples[1] | Present[2] | Absent[3] |
|---|---|---|---|
| **2d post-breeding** | 89 | 0 | 89 |
| **7d post-breeding[4]** | 166 | 0 | 166 |
| **90d post-breeding** | 71 | 0 | 71 |
| **112d post-breeding** | 17 | 0 | 17 |
| **Total** | **343** | **0** | **343** |

[1]The number of samples that were analyzed for the Tg in experiment 3 from C pigs after mating.
[2]The number of tissue samples that had the presence of Tg.
[3]The number of tissue samples that had the absence of the Tg.
[4]The 7 d post-breeding number includes the male and the female samples from experiment 3.
T=Transgenic pig, C=Control (non-transgenic) pig, Tg=transgene, d=day

**Table 6:** The number of tissue samples analyzed for parturition and gestation (Experiment 4a).

| | | | | Non-Transgenic Mating | | Transgenic[1] Mating | |
|---|---|---|---|---|---|---|---|
| Treatment | # of tissue samples[2] | # Non-Transgenic tissue samples[3] | # Transgenic tissue samples[4] | Present[5] | Absent[6] | Present[5] | Absent[6] |
| **Newborn-Non-suckle[7]** | 243 | 112 | 131 | 0 | 112 | 79 | 52 |
| **112d fetuses[8]** | 285 | 127 | 158 | 0 | 127 | 99 | 59 |
| **Total** | **528** | **239** | **289** | **0** | **239** | **178** | **111** |

[1]DNA was isolated from ear biopsies to determine that the Tg was present at the birth of these pigs.
[2]The total number of tissue samples that were T and non-T combined used in sub-experiment 4a.
[3]The number of tissue samples that were non-T used in sub-experiment 4a.
[4]The number of tissue samples that were T used in sub-experiment 4a.
[5]The number of tissue samples that had the presence of Tg.
[6]The number of tissue samples that had the absence of the Tg.
[7]Piglets that were allowed to farrow normally, but not allowed to suckle their birth dam.
[8]Fetuses that were collected at 112 d post-breeding.
Differential Tg expression between transgenic groups at 0 d and 112 d P-value < 0.0001.
T=Transgenic pig, C=Control (non-transgenic) pig, Tg=transgene, d=day

**Table 7:** Distribution of Tg among tissue samples from 112 d fetuses from heterozygous transgenic sows mated to non-transgenic boars (Experiment 4).

| Tissue Source | Specific Tissue Analyzed | Tg Present[1] | Tg Absent[2] |
|---|---|---|---|
| **Fetal[3]** | **Muscle** | 13 | 10 |
| | **Lung** | 13 | 10 |
| | **Liver** | 13 | 10 |
| | **Jejunum** | 13 | 10 |
| | **Internal Placenta** | 13 | 10 |
| | | | |
| **Maternal[4]** | **External Placenta** | 23 | 0 |

[1] The number of tissue samples that had the presence of Tg.
[2] The number of tissue samples that had the absence of the Tg.
[3] Tissue samples were derived from the fetus.
[4] Tissue samples were derived from the dam.
Abbreviations: T=Transgenic pig, C=Control (non-transgenic) pig, Tg=transgene, d=day

pigs; 2) is not transferred from transgenic pigs to the genome of non-transgenic pigs through direct contact; 3) is not transferred to the genome of control females or males through mating with a transgenic animal, and 4) is not transferred to the genome of a non-transgenic fetus during gestation in a transgenic mother, nor transferred to the genome of a non-transgenic neonate through suckling a transgenic sow. These results indicate that the transfer of genetic material from a transgenic animal to a non-transgenic animal does not result in alteration of the genome of the non-transgenic animal.

Lactogenesis involves the changes in the mammary epithelial cells that allow the development from the relatively undifferentiated mammary gland in pregnancy to full lactation sometime after parturition [27]. Lactogenesis in the porcine mammary gland, like other species, can be divided into two stages. Stage I (secretory differentiation) occurs starting between days 90 and 105 of gestation [28,29]. Stage II of lactogenesis (secretory activation), the onset of copious milk secretion, coincides with a rapid increase in milk volume and is thought to occur at approximately 34 hours postpartum in the sow [30,31]. Interestingly, in this study, there was no evidence showing that the Bα-LA Tg was expressed in the mammary gland of a sow at d 112 of gestation. We have previously seen the expression of the Bα-LA Tg at day 0 of lactation, shortly after parturition was completed [12]. This observation has also been made by others [32-34]. This gene was expressed, however, in the salivary gland of a transgenic animal. This expression profile is not surprising as both the mammary gland and the salivary glands are exocrine glands. As salivary glands and mammary glands share a similar ductulo-acinar architecture [35].

In swine production, pigs are regularly housed in groups, so it is imperative to determine if cohabitation of transgenic pigs with non-transgenic pigs results in the transfer of Tg among the pigs. This was done to look at the horizontal transmission of the Bα-LA Tg from one animal to the other though things like oral fluids, skin cells, tears, urine, and other bodily fluids. In this study, the pigs were able to perform typical behaviors like ingesting bodily fluids, licking, rubbing, and biting during cohabitation. There is evidence that pigs group-housed have a higher percentage of transmission of diseases such as Salmonella [36] than those that are individually housed. In contrast to the transmission of contagious pathogenic organisms, this study demonstrated that Bα-LA Tg was not horizontally transmitted among pigs during cohabitation. This study also evaluated the horizontal transmission of the Bα-LA Tg through reproduction and exposing the control pigs to Bα-LA Tg pigs during mating. This is a concern because it is likely that during copulation there is an exchange of cells from the genitals that could 'contaminate' the female reproductive tract with the Tg. Interestingly, this study did not find any evidence for horizontal transmission of the Bα-LA Tg. Ejaculated sperm and leukocytes found in semen are rapidly cleared from the reproductive tract and DNA from those cells does not become integrated as a result of horizontal transmission.

**Table 8:** The number of tissue samples analyzed for cross-fostering (Experiments 4b and 4c).

| Day | # of tissue samples[1] | Present[2] | Absent[3] |
|---|---|---|---|
| **d 0 cross-foster[4]** | 87 | 0 | 87 |
| **d 3 cross-foster[5]** | 70 | 0 | 70 |
| **Total** | **157** | **0** | **157** |

[1]The number of samples that were from non-T pigs and analyzed for experiments 4b and 4c.
[2]The number of tissue samples that had the presence of Tg.
[3]The number of tissue samples that had the absence of the Tg.
[4]Non-T piglet samples that were analyzed from piglets that were fostered to either a lactating T sow or a non-T sow immediately after birth before they were allowed to suckle their birth dam.
[5]Non-T piglet samples that were analyzed from piglets that were fostered to either a lactating T sow or a non-T sow at 3 d after birth.
T=Transgenic pig, C=Control (non-transgenic) pig, Tg=transgene, d=day

We observed that the external part of the placenta of non-transgenic fetuses carried by Tg female tested positive for the transgene, while the rest of the tissues tested negative. This is expected because the external part of the placenta, which is also referred to as the basal plate, represents the maternal surface of the placenta. The basal plate contains fetal trophoblasts and several maternal cell types that include the decidual stromal cells, natural killer cells, macrophages, and other immune cells [37]. These maternal cells from a T female are detected by the PCR analysis.

The fourth experiment examined whether the Tg was transferred to the piglet while suckling colostrum from a T sow or suckling milk from a T sow. Two features of this early neonatal period are important for considering the results of this experiment. First, intestinal macromolecular closure occurs in newborn piglets between 18 and 36 h after birth [38], coinciding with the period of high immunoglobulin concentrations in sow mammary secretions [39], thereby defining the colostrum phase of lactation. And, second, both colostrum and milk of the sow contain concentrations of leukocytes [40]. Despite the presence of leukocytes in the colostrum and milk ingested by piglets, the Tg was not identified in any tissues of cross-fostered piglets in Experiment 4.

Animals can be genetically modified to improve food animal production. Transgenic livestock can be used to improve milk production and composition, growth rate, feed utilization, disease resistance, reproductive performance, and prolificacy [2,41]. The improvement of the composition and nutrients of milk may have a great influence on the survival and growth of newborns in both humans and pigs. Low levels of milk production in sows is limiting to piglet growth and, therefore pig production [2,13,16]. A way to increase milk production in pigs may be achieved by changing the milk composition. We have previously shown that it is possible to increase milk production and piglet growth with these transgenic swine [2,13,16]. Lactose is a major osmole in milk. Lactose is formed inside the vesicles of the Golgi apparatus of mammary secretory cells, transported to the apical membrane of the epithelial cells via secretory vesicles and secreted into the lumen. Lactose draws water into the vesicles by osmosis, and therefore higher levels of lactose translate to higher volume of milk volume produced. Lactose is synthesized by the lactose synthase complex, which is composed of the mammary-specific protein α-LA and the enzyme α-1,4 galactosyl transferase. Because the complex is necessary for the production of lactose

and lactose is necessary for the movement of the water into the secretory vesicles and so into the lumen of the gland, it becomes evident that the complex is critical in the control of milk secretion. In particular, it was demonstrated that milk volume is directly related to the expression of the α-LA gene: α-LA gene expression correlates with the induction of copious milk secretion at the beginning of lactation [42].

To increase milk production in pigs we have previously produced two lines of transgenic pigs containing the Bα-LA gene [12]. The Bα-LA gene was chosen for a variety of reasons, such as its expression is the most strictly regulated and lactation specific of all the bovine milk protein genes; it is produced in the milk of most animals, and the bovine and porcine α-LA proteins have similar molecular weights [2]. We have shown that the Bα-LA gene can be expressed in the pig, and the protein can be secreted into the milk. The concentration of Bα-LA was highest on d 0 of lactation and decreased as lactation progressed [20]. Because Bα-LA was being produced at higher levels at the beginning of lactation while porcine α-LA had not yet reached its maximum concentration, the total α-LA of transgenic sows was dramatically elevated in early lactation milk. The production of the bovine protein caused approximately a 50 % increase in the total α-LA concentration of pig milk throughout lactation. Interestingly, the level of milk protein and total solids was not significantly affected by the increased lactose concentration on d 0. If a higher lactose concentration would lead to increased milk production, one may expect that the concentration of protein and total solids would be lower due to added water being drawn into milk by osmosis, but this was not the case. The mammary gland machinery appeared to be able to re-equilibrate the milk composition in the right amount of solids, protein, and water.

It is worth pointing out here that the goal of using this technology is for the benefit, not the detriment of mankind. The use of this technology is not simple, efficient, or inexpensive. Scientists using this technology are trying to develop models to study disease, produce biopharmaceuticals, and produce more wholesome, healthy, and economical food. These studies are difficult and great care must be taken before such investigations begin. Such considerations are critical due to the time, cost, welfare, ethics, concerns, risks, and benefits involved in these kinds of investigations. None of these groups, producers, consumers, or scientists, are motivated to produce inappropriate medical models, ineffective or dangerous pharmaceuticals, or unsafe food. None of these groups would survive the political and economic repercussions if this were the case. Therefore, although this type of research is expensive and challenging to implement, concern for animal welfare, ethics, societal benefit, and vigilance should be emphasized that much more during the development of research objectives. Consideration of these, as well as scientific issues, will lead us forward to reaping the benefits of this transformative technology.

## CONCLUSION

It is clear that for the long-term benefit of society and the area of transgenic technology, the impacts on the environment, producers, consumers, and especially the animals must be carefully evaluated. This opinion has already been presented to the scientific community, and it is important for scientists using this technology to become engaged and be willing participants in the discussion and consideration of ethical issues, concerns and potential risks surrounding the implementation of this work [43].

## ETHICAL APPROVAL

All procedures performed in studies involving animals were in accordance with the ethical standards of the University of Illinois Institutional Animal Care and Use Committee, (IACUC Approval #07190). This article does not contain any studies with human participants performed by any of the authors.

## ACKNOWLEDGEMENT

The authors would like to thank Dr. Michael A. Cotta-USDA ARS-Peoria, IL, Dr. Elisa Monaco, Dr. Alecsandra Lima, Ms. Meredith Cake, Ms. Tara VanEtten, Ms. Shanna Wilson, Ms. Synthia Lane and Mr. Glenn Bressner for thoughtful discussion, sample collection and analysis and exceptional care of the research animals. This work was funded by the USDA BRAG grant (#AG 2005-39454-16435) and the Council for Food and Agricultural Research of the State of Illinois (C-FAR #97-136 and Sentinel Grant Program).

## REFERENCES

1. Wall RJ, Seidel GE Jr. Transgenic farm animals - A critical analysis. Theriogenology. 1992; 38: 337-357.
2. Wheeler MB. Production of transgenic livestock: promise fulfilled. J Anim Sci. 2003; 81 Suppl 3: 32-37.
3. Tumbleson ME, Schook LB. Advances in swine in biomedical research. Volume 1. In: Tumbleson ME, Schook LB, editors; 1996 October 22-25, 1995; University of Maryland, College Park, MD. Plenum Press, 233 Spring Street, New York, NY 10013. pp. xxxix + 422.
4. Kumar M, Ratwan P. Transgenic or Genetically Modified farm animals and their applications: A Review. Journal of Veterinary Science and Technology. 2016; 5: 25-34.
5. Singh B, Mal G, Gautam SK. Reproduction Biotechnology in Pigs. Advances in Animal Biotechnology. Cham: Springer International Publishing. 2019; 169-182.
6. Murray JD, Maga EA. Is there a risk from not using GE animals? Transgenic Research. 2010; 19: 357-361.
7. Liu S, Li C-X, Feng X-L. Safety assessment of meat from transgenic cattle by 90-day feeding study in rats. Food and Chemical Toxicology. 2013; 57: 314-321.
8. Clark M, Murray JD, Maga EA. Assessing unintended effects of a mammary-specific transgene at the whole animal level in host and non-target animals. Transgenic Research. 2014; 23: 245-256.
9. Tang M, Zheng X, Cheng W. Safety assessment of sFat-1 transgenic pigs by detecting their co-habitant microbe in intestinal tract. Transgenic Research. 2011; 20: 749-758.
10. Van Reenen CG, Meuwissen THE, Hopster H, Oldenbroek K, Kruip Th AM, Blokhuis HJ. Transgenesis may affect farm animal welfare: a case for systematic risk assessment. Journal of Animal Science. 2001; 79: 1763-1779.
11. Murray JD, Maga EA. Opinion: A new paradigm for regulating genetically engineered animals that are used as food. Proceedings of the National Academy of Sciences. 2016; 113: 3410-3413.
12. Bleck GT, White BR, Miller DJ, Wheeler MB. Production of bovine alpha-lactalbumin in the milk of transgenic pigs. J Anim Sci. 1998; 76: 3072-3078.
13. Noble MS, Rodriguez-Zas S, Cook JB, Bleck GT, Hurley WL, Wheeler

MB. Lactational performance of first-parity transgenic gilts expressing bovine alpha-lactalbumin in their milk. J Anim Sci. 2002; 80: 1090-1096.

14. Grimble RF, Mansaray YKC. Effects in Rats of Dietary Protein Inadequacy on Lactose Production, Milk Volume and Components of the Lactose Synthetase Complex (EC 2.4.1.22). Annals of Nutrition and Metabolism. 1987; 31: 179-184.

15. Kuhn NJ, Carrick DT, Wilde CJ. Lactose Synthesis: The Possibilities of Regulation. Journal of Dairy Science.1980; 63: 328-336.

16. Marshall KM, Hurley WL, Shanks RD, Wheeler MB. Effects of suckling intensity on milk yield and piglet growth from lactation-enhanced gilts. Journal of Animal Science. 2006; 84: 2346-2351.

17. Boyd DR, Kensinger RS. Metabolic precursors for milk synthesis. In: Verstegen MWA, Moughan PJ, Scharma JW, editors. The Lactating Sow: Wageningen Press, Wageningen. 1998; 71-95.

18. Hogan B, Constantini F, Lacey E. Manipulating the Mouse Embryo: A Laboratory Manual. . Cold Spring Harbor, NY: Cold Spring Harbor Laboratory. 1986.

19. Marcia H Monaco, Derek E Gronlund, Gregory T Bleck, Walter L Hurley, Matthew B Wheeler, Sharon M Donovan. Mammary specific transgenic over-expression of insulin-like growth factor-I (IGF-I) increases pig milk IGF-I and IGF binding proteins, with no effect on milk composition or yield. Transgenic Research. 2005; 14: 761-773.

20. Bleck GT, Bremel RD. Variation in expression of a bovine alpha-lactalbumin transgene in milk of transgenic mice. J Dairy Sci. 1994; 77: 1897-1904.

21. Bialek R, Feucht A, Aepinus C, Gudrun Just-Nübling, Valerie J Robertson, Jürgen Knobloch, et al. Evaluation of two nested PCR assays for detection of Histoplasma capsulatum DNA in human tissue. Journal of clinical microbiology. 2002; 40: 1644-1647.

22. Noyes HA, Reyburn H, Bailey JW, Smith D. A Nested-PCR-Based Schizodeme Method for Identifying Leishmania Kinetoplast Minicircle Classes Directly from Clinical Samples and Its Application to the Study of the Epidemiology of Leishmania tropica in Pakistan. Journal of Clinical Microbiology. 1998; 36: 2877.

23. Stabel JR, Bannantine JP. Development of a nested PCR method targeting a unique multicopy element, ISMap02, for detection of Mycobacterium avium subsp. paratuberculosis in fecal samples. Journal of clinical microbiology. 2005; 43: 4744-4750.

24. Maschio A , Brickell P M , Kioussis D , Mellor A L , Katz D , Craig R K. Transgenic mice carrying the guinea-pig α-lactalbumin gene transcribe milk protein genes in their sebaceous glands during lactation. Biochem J. 1991; 275: 459-467.

25. Lipsey MW. Design Sensitivity: Statistical Power for Experimental Research: SAGE Publications. 1990.

26. SAS (2018) SAS OnlineDoc®. 15.1 ed. Cary, NC: SAS Institute Inc.

27. Neville MC, Morton J, Umemura S. Lactogenesis: The Transition from Pregnancy to Lactation. Pediatric Clinics of North America. 2001; 48: 35-52.

28. Kensinger RS, Collier RJ, Bazer FW. Ultrastructural changes in porcine mammary tissue during lactogenesis. J Anat. 1986; 145: 49-59.

29. Kensinger RS, Collier RJ, Bazer FW, Ducsay CA, Becker HN. Nucleic acid, metabolic and histological changes in gilt mammary tissue during pregnancy and lactogenesis. Journal of animal science. 1982; 54: 1297-1308.

30. Krogh U, Flummer C, Jensen SK, Theil PK. Colostrum and milk production of sows is affected by dietary conjugated linoleic acid. J Anim Sci. 2012; 90: 366-368.

31. Theil PK, Lauridsen C, Quesnel H. Neonatal piglet survival: impact of sow nutrition around parturition on fetal glycogen deposition and production and composition of colostrum and transient milk. Animal. 2014; 8: 1021-1030.

32. Nicholas KR, Hartmann PE, McDonald BL. Alpha-Lactalbumin and lactose concentrations in rat milk during lactation. Biochemical Journal. 1981; 194: 149-154.

33. Quirk SJ, Slattery JA, Funder JW. Mammary gland whey acidic protein: ontogeny and changing patterns of steroid sensitivity. Molecular and Cellular Endocrinology. 1989; 66: 135-141.

34. Vilotte JL, Soulier S, Printz C, Mercier JC. Sequence of the goat α-lactalbumin-encoding gene: comparison with the bovine gene and evidence of related sequences in the goat genome. Gene. 1991; 98: 271-276.

35. Skálová A, Tomas Vanecek, Radek Sima, Jan Laco, Ilan Weinreb, Bayardo Perez-Ordonez, et al. Mammary Analogue Secretory Carcinoma of Salivary Glands, Containing the ETV6-NTRK3 Fusion Gene: A Hitherto Undescribed Salivary Gland Tumor Entity. Am J Surg Pathol. 2010; 34: 599-608.

36. Wilkins W, Rajić A, Waldner C, McFall M, Eva Chow, Anne Muckle, et al. Distribution of Salmonella serovars in breeding, nursery, and grow-to-finish pigs, and risk factors for shedding in ten farrow-to-finish swine farms in Alberta and Saskatchewan. Can J Vet Res. 2010; 74: 81-90.

37. Huppertz B. The anatomy of the normal placenta. Journal of Clinical Pathology. 2008; 61: 1296-1302.

38. Westrom BR, Svendsen J, Ohlsson BG, Tagesson C, Karlsson BW. Intestinal transmission of macromolecules (BSA and FITC-labelled dextrans) in the neonatal pig. Influence of age of piglet and molecular weight of markers. Biology of the Neonate. 1984; 46: 20-26.

39. Jackson JR, Hurley WL, Easter RA, Jensen AH, Odle J. Effects of induced or delayed parturition and supplemental dietary fat on colostrum and milk composition in sows. Journal of animal science. 1995; 73: 1906-1913.

40. Hurley WL, Grieve RC. Total and differential cell counts and N-acetyl-beta-D-glucosaminidase activity in sow milk during lactation. Vet Res Commun. 1988; 12: 149-153.

41. Wheeler MB. Transgenic Animals in Agriculture. Nature: Education Knowledge. 2013; 4: 1-10.

42. Goodman RE, Schanbacher FL. Bovine lactoferrin mRNA: sequence, analysis, and expression in the mammary gland. Biochem Biophys Res Commun. 1991; 180: 75-84.

43. Mench JA. Ethics, animal welfare and transgenic farm animals. In: Murray JD, Anderson GB, Oberbauer AM et al., editors. Transgenic Animals in Agriculture: CABI International. 1999; 251-268.

**Cite this article**

*Mosley JF, Hurley WL, Rodriguez-Zas SL, Wheeler MB (2020) Evaluation of Risks from Environmental Contact with Transgenic Livestock. J Vet Med Res 7(3): 1190.*