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Full Length Research Paper

Sonographic evidence of follicle development in a fixed time AI synchronization protocol involving ovatide in Bunaji cows

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An investigation was done to observe follicle development and ovulation by ultrasound in a synchronization protocol in Bunaji cows using ovatide. Cows (n=16), aged 4 to 6 years with average body condition scores of 2.5 to 3.5 and weighing between 250 and 350 kg were used. They were managed according to the routine management practice of the Diary Research Programme NAPRI. Only cycling cows at 75 days post-partum with palpable CL were included in the study. Cows were randomly assigned to 1 or 2 treatment groups for synchronization of ovulation. Treatment group 1 comprising Bunaji (n=8) received 50 µg of GnRH and 25 mg of $PGF_{2\alpha}$. While, treatment group 2 comprising Bunaji (n=8) received 50 µg of ovatide and 25 mg of $PGF_{2\alpha}$. The treatment was as follows: Group 1: (Day 0, 50 µg GnRH; Day 7, 25 mg $PGF_{2\alpha}$ and day 9, 50 µg GnRH), group 2: (Day 0, 50 µg ovatide, Day 7, 25 mg $PGF_{2\alpha}$ and Day 9, 50 µg ovatide). Ultrasound examinations were conducted. Examinations were conducted at the time of second gonadotropin injections, to determine presence of one or more antral follicles > 10 mm in diameter and at 48h after second gonadotropin injections, to determine absence of 1 (single – ovulation) or 2 (double – ovulation) of those earlier antral follicles. Results showed synchronization rate for ovatide was 75%, while that of GnRH (Cystorellin) was 62.5% (p>0.05). Double ovulation rate for both groups was 0%. It was concluded that 50 µg Ovatide in Ovsynh protocol has synchronization potentials in Bunaji cows. Further studies on gonadotropins of fish origin are recommended.

Key words: Ovatide, follicle, sonographic, synchronization, Bunaji, cows.

INTRODUCTION

Ovatide is an indigenous, cost-effective and new hormonal formulation for induced breeding of fishes. It is also effective in breeding major carps. The dosages for females are 0.20 to 0.40 ml/kg for rohu and mrigal, 0.40 to 0.50 ml/kg for catla, silver carp and grass carp, the dosages for males are 0.10 to 0.20 ml/kg for rohu, mrigal, 0.20 to 0.30 ml/ kg for catla and 0.20 to 0.25 ml/kg for silver carp and grass carp (Naipagropediaraichur, 2012). It is a new, highly potent and ready to use injectable formulation containing a synthetic peptide analogous to the naturally occurring hormone, salmon GnRH. The formulation also contains a dopamine antagonist, whereas the GnRH analogue stimulates the pituitary to release gonadotropins and trigger the process of

reproduction, the dopamine antagonist inhibits the release of dopamine and makes sure that the secretion of gonadotropins is not inhibited. The use of Ovatide, thus constitutes the latest and the most advanced technology employed for induced breeding of fishes and production of high quality fish seed. It is composed of Gonadorelin A (GnRH A) 20 mcg, Domperidone BP 10 mg and benzyl alcohol IP 1.5% v/v (HemoPharma, 2014).

Gonadotropic releasing hormone (GnRH) is labeled for treating follicular cysts in cows at a dosage of 100 µg (Merial Animal Health, Duluth, GA) in the United States. This is why the Ovsynch protocol utilizes a 100 µg dose. Administration of 100 µg GnRH after $PGF_{2\alpha}$ injection increases the rate of synchronized ovulation in bovines (Pursley et al., 1995). A study by Navanukraw et al., (2002) reported a 37.5% pregnancy rate at 42 post AI using Ovsynch with two half dose of GnRH. Also, Fricke et al., (2003) found similar pregnancy rates using half dose Ovysynch on second service animals.

Another study conducted using Holstein Friesian cows in Wisconsin compared 100 µg dose of GnRH to lower 50 µg dose and reported no statistical difference between treatment groups (Fricke et al., 1998). Thus, 50 µg dose of GnRH decrease total hormone cost from $16. 10 to $9.70 and the total cost per pregnancy was reduced from $47.88 to $27.61, making it comparable to the cost of $PGF_{2\alpha}$ only program (Fricke et al., 1998). Because of the increasing demand for the application of AI in Nigeria's indigenous cattle breeds and the need for fixed timed AI, the cost of using conventional hormones plays a negative role in disseminating the technology and making it available to poor farmers. The application of ovatide, which is a synthetic analogue of GnRH of fish (Salmon) origin in a synchronization protocol in bovines, may be a potential relief from the exorbitant cost of imported hormones. If cows respond to ovatide well in a synchronization protocol, there will be hope that the hormone extracted from live catfish can replace the expensive analogues. The pituitary of live catfish can be harvested from fish processing plants, currently the bye products of such processing plants are being wasted in our society. This work is designed to investigate follicle development and ovulation by ultrasound in a synchronization protocol in Bunaji cows using ovatide.

MATERIALS AND METHODS

Study location

This study was carried out at the cattle farm of Diary Research Programme (DRP) of the National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria. Shika is situated in the Nothern Guinea Savannah between Latitudes 11° and 12° and Longtitudes 7° and 8° E at elevation of 659 m above sea level with an average annual maximum and minimum temperature of 31.0± 3.2 and 18.0±3.7°C, respectively. It has two distinct seasons: dry season (November to April) with mean daily temperature ranging from 15 – 36°C and rainy season (May to October) with average annual rainfall of 1100 mm and mean relative humidity of 72% (Rekwot et al., 1998).

Research animals and management

The animal experiments followed the principles of the laboratory animal care (CACC, 1993). Bunaji cows (n=16) aged 4 to 6 years and weighing between 250 to 350 kg were used. Selected cows with average body condition scores (BCS) of 2.5 to 3.5 using 0 to 5 scale from the most emaciated to the fattest (Pullman, 1978). Cows were identified by means of plastic ear tags. They were managed according to the routine management practice of the Diary Research Programme. Two transrectal examinations a month apart were carried out to ensure cyclicity of the cows before commencement of the study. Only cycling cows at 75 days post-partum with palpable CL were included in the study.

Experimental design

Cows were randomly assigned to 1 or 2 treatment groups for synchronization of ovulation. Treatment group 1 comprising Bunaji (n=8) received 50 µg of GnRH (Cystorellin; Nerial, Ltd., Iselin, NJ) and 25 mg of $PGF_{2\alpha}$ (Lutalysethe Pharmacia – Upjohn Co., Kalamazoo, MI). While, treatment group 2 comprising Bunaji (n=8) received 50 µg of ovatide (Hemo Pharm. PVT Ltd., Mahalaxmi, Mumbai) and 25 mg of $PGF_{2\alpha}$. The treatment is as illustrated below.

Treatment Group 1

Day 0, 50 µg GnRH; Day 7, 25 mg $PGF_{2\alpha}$ and day 9, 50 µg GnRH. Bunaji cows administered first injection of 50 µg of GnRH on the day 0, followed by 25 mg of $PGF_{2\alpha}$ 7 days later and a second injection of 50 µg GnRH 48 h after $PGF_{2\alpha}$ administration (Figure 1).

Treatment group 2

Day 0, 50 µg Ovatide; Day 7, 25 mg $PGF_{2\alpha}$ and Day 9, 50 µg ovatide. These cows were administered first injection of 50 µg ovatide on day 0, followed by 25 mg of $PGF_{2\alpha}$ 7 days later and a second injection of 50 µg ovatide 48 h after $PGF_{2\alpha}$ administration (Figure 2)

Ultrasonography and rectal palpation

Ultrasound examinations were conducted using ultrasound machine equipped with a transrectal 7.5 MHz linear – array transducer (Aloka 500v; Corometrics Medical Systems, Inc., Willing Ford, (CT). Examinations were conducted at the time of second GnRH injection, to determine presence of one or more antral follicles > 10 mm, in diameter and at 48 h after second GnRH injection, to determine absence of 1, (single – ovulation) or 2 (double –

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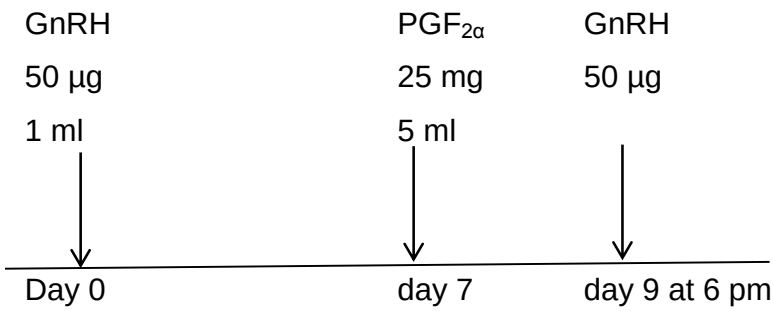

Figure 1. Treatment days and doses with Cystorelin and PGF2α.

ovulation) of those earlier antral follicles.

Transrectal palpation was conducted twice at a month interval to select cycling cows within 5 to 12 days of the estrous cycle before initiating Ovsynch protocol (Voh, 1997).

Fertility rates

Synchronization rate

Synchronization rate was calculated as the number of cows that ovulated at least 1 follicle within 48 h of the second GnRH injection, expressed as a percentage of the total number of cows that received the Ovsynch protocol.

Double ovulation rate

Double- ovulation rate was calculated as the number of cows that ovulated 2 follicles within 48 h of the second GnRH injection, expressed as a percentage of synchronized cows.

Statistical analysis

Data obtained on follicle development, synchronization rate, double ovulation rate were expressed in percentages and represented in charts. Differences in the parameters between treatment groups were analyzed using Chi-squares test. Values of $P < 0.05$ were considered significant. A data analysis was carried out using Statistical Package for Social Sciences (SPSS) Version 17.0.0 (SPSS Inc. Chicago IL, USA).

RESULTS

Synchronization rate (SR)

SR = No. of ovulations 48 h after 2ndGnRH/total no. of animals that received the treatment.
Ovatide = 6/8 x100 = 75%; GnRH = 5/8 X100 = 62.5%; P = 1.00, O.R = 1.80.

Double ovulation rate (DR)

DR = No. of cows that ovulated 2 follicles within 48 h of the 2ndGnRH injection divided by the number of synchronized cows. Ovatide = 0.6 x100 = 0%; GnRH = 0.5 x100 = 0%.

DISCUSSION

The use of 50 µg of ovatide in the fixed time artificial insemination synchronization protocol showed that there was adequate follicular development (Figure 4 and Plate 1). The GnRH (Cystorelin) at 50 µg also showed adequate follicular development by day nine just before the 2nd gonadotropin injections (Figure 4 and Plate 2). This means that the bunaji cows responded equally to both treatments in respect of follicle development. This response may be complementary to prostaglandin injections on day seven. Subsequently, the number of cows that ovulated 48 h following 2nd gonadotropin injections did not differ significantly between the two groups ($P>0.05$), this means that both treatments were capable of luteinizing the developed follicles at the treatment doses of 50 µg. A synchronization rate of 75% was recorded for the ovatide while 62.5% was recorded for the GnRH (Cystorelin) (Figure 3). Synchronization rate between the two groups did not differ significantly. The implication of this is that ovatide has synchronization potentials in the Bunaji cows just like Cystorelin.

The emergence of a new follicular wave is synchronized only when GnRH treatment causes ovulation (Martinez et al., 1999). If the first GnRH does not synchronize follicular wave emergence, ovulation following the second GnRH may be poorly synchronized (Martínez et al., 2002a), resulting in disappointing pregnancy rates following TAI (Martínez et al., 2002b). The ovatide showed a higher value of synchronization rate (75%) as compared to Cystorelin (62.5%), this may be attributed to factors such as poor compliance and dose.

Anytime you add another cow handling to a program, you are likely increase the probability that not every cow will be treated with the right product at the right time. Keeping things simple and understandable for everyone involved in the breeding program is priority. Knowing the difference between products to be used, using proper syringe and needle sizes (18 g, 1.5 inch), and following

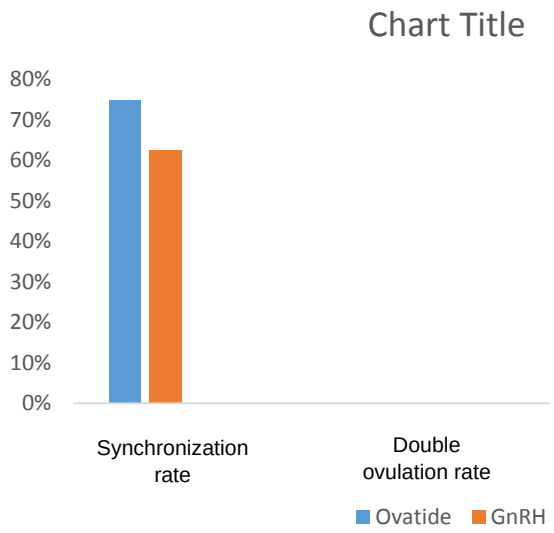

Figure 3. Synchronization and double ovulation rates of ovatide and GnRH (Cystorelin) following the Ovsynch protocol.

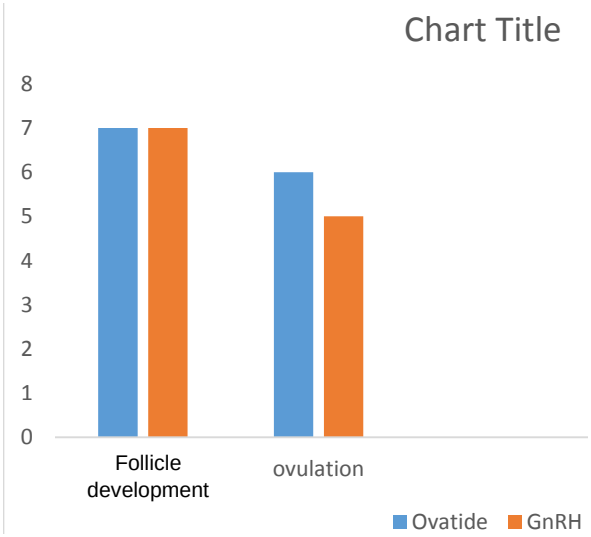

Figure 4. Parameters measured following treatment and sonography. P=1.00, O.R= 0.4286; P=1.00, O.R =1.80; O.R = Odds ratio.

instructions are key to a high rate of compliance and good AI conception rates (Jeff, 2016). The recommended dose of Cystorelin for cows is 100 µg. In this study, using 50 µg may have contributed to the lower synchronization rate of 62.5% as compared to 75% of ovatide. It has been reported that the dose of ovatide used in fish affects fertility parameters in fish. A study was conducted to evaluate ovatide doses (0.6, 0.8 and 1.0 ml/kg body weight of female) on breeding performance of *Clarias batrachus* in the subtropical region of Hisar. The breeding

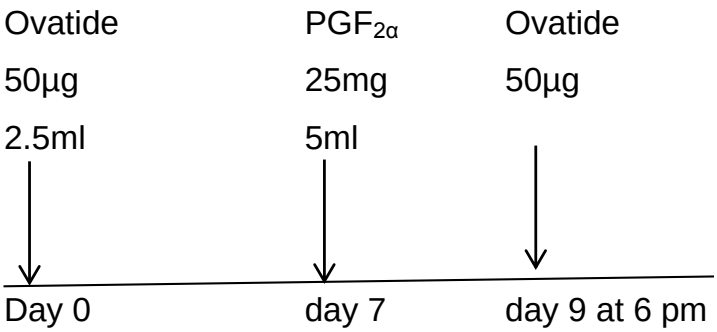

Figure 2. Treatment days and doses with Ovatide and PGF2α.

performance was judged on the basis of the total weight of stripped eggs, net fecundity, fertilization, hatching and survival. To judge the egg quality, the per cent fertilization, hatching and survival of fry were considered. The results indicated that the total weight of stripped eggs and spawning fecundity were the highest ($p < 0.05$) when females were injected with 1 ml of ovatide per kg body weight (BW) as compared to those injected with other dose levels. The lowest stripping response was observed with injection of 0.6 ml ovatide per kg BW of female brood fish. At the 1 ml dose, the percentages of total fertilized egg and hatching were 82.33 and 55.35% respectively, which were the highest ($p < 0.05$) among all treatments. The net survival of fry was found to be 98.52% at 1 ml ovatide per kg BW. Therefore, it has been recommended that 1 ml of ovatide per kg BW of female brood fish was found optimum among the three experimental doses for best breeding performance and egg quality in *C. batrachus* (Sharm et al., 2010). Similarly, GnRH is recommended for treatment of follicular cysts at 100 µg. This report means that there is an optimum dose of gonadotropin (Ovatide or Cystorelin) that is required for ovulation to occur in a particular species as reflected in the total number of stripped eggs in fish.

Conclusions

Both ovatide and GnRH (Cystorelin) showed sonographic evidence of follicular development on day nine of the treatments, before the 2nd gonadotropin injections. Ovulations occurred in both treatment groups which were not significantly different. A synchronization rate of 75 and 62.5% were recorded for ovatide and GnRH (Cystorelin), respectively. It was concluded that treatment of bunaji cows with 50 µg ovatide in Ovsynh protocol has synchronization potentials. Based on the outcome of this study, it was recommended that further studies be carried out using pituitary extract of *C. gariepinus* (African catfish) in a fixed time AI synchronization protocol in bunaji cows.

Plate 1. Sonographic evidence of antral follicle on the ovaries before 2nd ovatide injection.

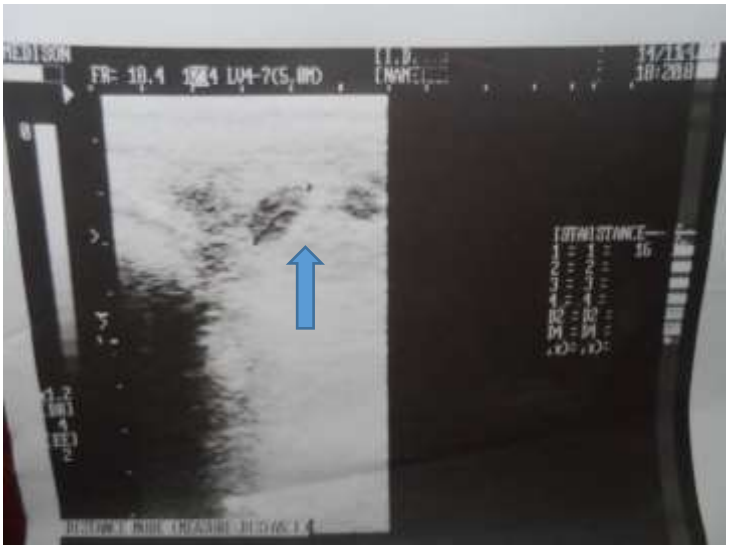

Plate 2. Sonographic evidence of antral follicle on the ovaries before 2nd GnRH (Cystorelin) injection.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

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Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Semen characteristics and fertility assessment of *Sennar* jackass *(Equus asinus)* in Ethiopia

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The aim of this study was to evaluate the semen characteristics and fertility of *Sennar* jacks. Semen was collected between January and March using Missouri AV model and was subjected to gross and microscopic evaluation. Fertility was evaluated from pregnancy rate after AI was carried out with fresh semen on 12 Abyssinian jennies, 5 *Sennar* jennies and 17 local mares. Mean (±SD) total and gel-free volume; and spermatozoa concentration were 61.1±12.6 ml, 50.3±12.3 ml, and 257± 8.1 x 10^6/ml, respectively. Total and progressive sperm motility, sperm viability and abnormal sperm percentage were 84.2±2.1; 67.4 ± 6.1%, 89%, and 10.9±2.9, respectively. There was no significant individual difference in most semen parameters. Pregnancy rate was 40% (2/5) in *Sennar* jennie, 58.3% (7/12) in Abyssinian jennies and 64.7% (11/17) in mares. The study thus revealed that semen can be successfully collected and evaluated as part of a breeding soundness examination of *Sennar* jacks during cross breeding using AI. Further study on cryopreservation of semen and improving pregnancy rate after extending semen in optimized donkey semen extender is necessary in the future.

Key words: AI, fertility, *Sennar* jacks, semen, Ethiopia.

INTRODUCTION

With an estimated 6.2 million heads (CSA, 2011), donkeys are known to play a great role particularly in rural areas of Ethiopia, where they are used on a daily basis to carry out numerous tasks in the house and agricultural fields (Alemu et al., 1997). Classifications of Ethiopian donkeys based on size and coat colour, includes four major types namely *Abyssinian, Jimma, Ogaden,* and *Sennar* Donkeys (Fesseha, 1991). The *Sennar* donkey is by far the largest and the only one reputed for producing good mules. The natural habitat of the *Sennar* donkeys is the Northwestern lowland of Ethiopia. Donkey crossing with selected descendants of the Kessella Nubian asses is a very common practice, while mule production from Sudan *Sennar* donkeys is more common in the highlands around Ethio-Sudan border. Mating, in almost all cases, except for the crossing, is uncontrolled and hence usually associated with year-round foaling.

Due to their distinct phenotypic features, *Sennar* donkeys are expensive and are not easily available in all

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areas of the country. Moreover, anecdotal information suggests that the work performance of *Sennar* donkeys is nearly twice that of Abyssinian types. Regardless, there is little published information on their genetic potential, selection, and reproductive management. Particularly, no work has been initiated to consider the use of AI, a technology which is relatively well developed in horses (Squires, 2005). At natural mating, the average fertile jack ass ejaculates 3.3-18 billion spermatozoa directly into the body of the uterus. Fewer than 100 spermatozoa pass through the uterotubal junction to reach the site of fertilization to give a per cycle conception rates of 60 – 70% (Allen et al., 2001; Hagstrom, 2004). AI in donkeys can improve the reproductive performance; however, expanded use of frozen semen is dependent on proper laboratory assessment of sperm quality as an essential procedure of the AI technology. Mares bred with frozen semen are often examined 4-6 times/day and inseminated immediately before or within 6 h post-ovulation because of lower survivability of spermatozoa in the reproductive tract (Squires et al., 2003; Contri et al., 2012). Another study (Samper, 2005) has shown that deep insemination into the horn ipsilateral to the ovary with the pre-ovulatory follicle results in 80% of the sperm remaining in that oviduct with-higher conception. On the other hand, with the apparent differences from horses, the efficient application of AI in donkeys requires an understanding of peculiar semen characteristics (Rota et al, 2012; Qeusada et al., 2012). This study was aimed at assessing *Sennar* donkey semen characteristics and evaluation of fertility of fresh ejaculate through AI.

MATERIALS AND METHODS

Animals

A total of 5 *Sennar* jacks previously selected for breeding at *Sennar* Donkey Multiplication Centre (Wekin, North Ethiopia) were used. The jacks were aged between 6 and 8 years, and had an average BCS of 7 (on 1-9 scale, Pearson and Quasat, 2000) body weight ranging 243 to 280 kg. The jacks were mainly used to produce donkey crosses and mules. Study jacks were allowed to graze in the field freely and were supplemented with ample amount of hay and concentrate. Water was provided *ad libitum*. All jacks were dewormed against common parasites, and vaccinated against African Horse Sickness and Anthrax before introduction to the stable.

Collection and evaluation of semen

The jacks were allowed to individually interact with jennies well into oestrus for 30 min. Semen was collected after the jacks were sufficiently stimulated using a Missouri model equine AV (Agatech, Manhattan, USA) twice a week for a total of 25 collections between January and March. Immediately after collection, the color and the total volume of the each ejaculate was recorded. Semen was then filtered and the gel-fraction removed and placed in water bath at 37°C. Aliquot of 5 μl semen was removed from the gel-free fraction for each of the following microscopic evaluation: total motility, progressive motility and sperm viability, abnormal sperm percent, pH was determined using strip. Sperm concentration (10^6/ml) was measured using Neubauer hemocytometer. Total and progressive motility were evaluated by phase contrast microscopy at ×100 and ×200 magnifications. The differential staining was made after taking a 10 μl aliquot of the semen sample mixed at 1:1 ratio of with 3% sodium citrate buffer and 1% eosin and made into thin smear. The slide was quickly dried on a pre-warmed plate before evaluation. Live percent and percent abnormal sperm were determined under light microscope (oil immersion, x1000) after counting 200 spermatozoa. White (unstained) sperm was classified as live and those that showed pink or red coloration were classified as dead. Morphological defects were classified into head, mid-piece or tail defects.

Fertility assessment

Fresh semen was extended in 1:1 volume by volume ratio of semen and a modified equine semen extender (prepared from 100 ml skimmed milk, 2.5 ml egg yolk, 4 g of glucose, 150,000 IU crystalline penicillin and 150,000 μg streptomycin) (Davies-Morel, 1999). Sperm longevity was evaluated at 15 min intervals for the first one hour, followed by final evaluation after one hour. The semen was then evaluated again after 24 h of chilling in Equitainer-I Tube Style (Agtech, Inc., Manhattan, U.S.A.) at +6°C. Spermatozoa fertilizing ability was afterwards determined by inseminating 17 jennies (5 *Sennar* jennets, 12 Abyssinian jennets) and 17 mares that were induced for estrus prior to AI. Both the jennies and mares used for AI were selected based on their previous history of breeding and each received 1ml prostaglandin (Clorprostenol, Pharmacia and Upjohn Company, USA) for induction of estrus. Jennies and mares were followed for manifestation of estrus signs. Semen used for insemination was collected the day of AI, prepared to make up $100x10^6$/ml (4 ml of semen; $400x10^6$ sperm per insemination) Insemination was carried out after ovulation (but as close to time of ovulation as possible) which was determined by ultrasound. A catheter was inserted per vaginum into the uterus and semen was deposited using a plunger free syringe. Small amount of air was pushed into the catheter to gently drive out the remaining semen. Pregnancy was diagnosed using ultrasonography (Mindray, Hong Kong) after 30 days post insemination. Fertility assessment was performed from pregnancy rate per insemination.

Statistical analysis

All collected data was stored in Microsoft Excel data sheet. The statistical analysis was performed using SPSS for Windows (Version-15) and STATISTICA for Windows (version 6, Statsoft, USA). The data was summarized using descriptive statistics. Comparison between jacks in fresh semen parameters was done using One Way ANOVA. Correlation between variables was computed using Pearson correlation (r). Differences were considered significant when $P< 0.05$.

RESULTS

A total of 25 semen collection procedures were carried out. Summary of fresh semen parameters are given in Table 1. The most prominent pre-coital sexual displays observed during teasing included nibbling and/or sniffing of the vulva, head, neck and back of the knee, flank, perineum and tail; Olfactory investigation of voided urine, flehman response, mounting with and without erection (Figure 1) and naso-nasal contact. Once a jack gets

Table 1. Fresh semen characteristics in *Sennar* jacks (n=25 collections).

Parameter	Mean (±SD)	Range
Total semen volume (ml)	61.1 ± 12.6	25 - 100
Gel-free volume (ml)	50.3 ± 12.3	15 - 85
pH	7.4 ± 0.1	6.5 – 7.7
Semen concentration (10^6/ml)	257 ± 8.1	65 – 387
Total sperm motility (%)	84.2± 2.1	78 - 92
Progressive sperm motility (%)	67.4 ±6.1	60 - 75
Sperm viability (%)	89.1 ± 2.3	82 – 94
Morphologically normal sperm (%)	89.0 ± 2.9	80 - 94

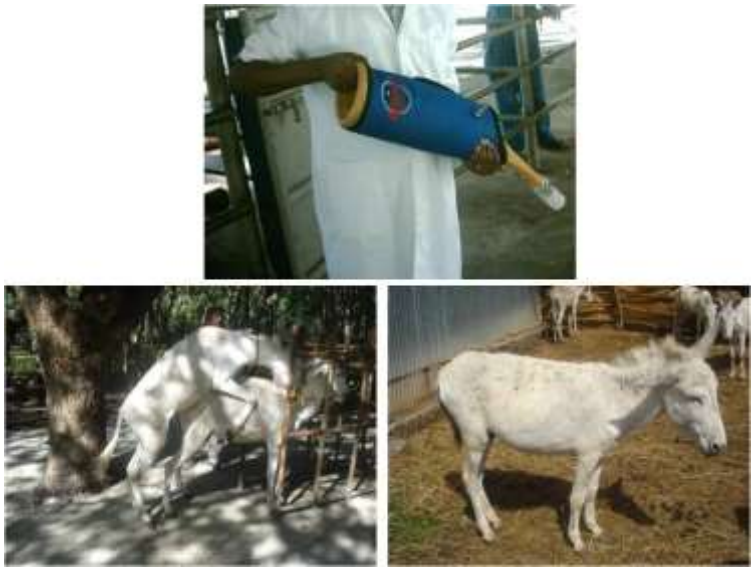

Figure 1. Semen collection in *Sennar* donkeys. Top: Missouri model equine AV used to collect *Sennar* jacks (Bottle cover removed), Left: False mount without erection during teasing, Right: Jack after semen collection displaying refractory isolation from estrous jennies.

erection, then it took on average less than two minutes to reach full erection and eventually being collected.Semen was collected successfully from all males on all occasions.Jacks entered into refractory period and often isolated themselves from the jenny in estrus after ejaculation.

Semen color was creamy white (91.5%), or milky (85%). There was no significant difference in semen parameters among individual jacks except for pH of semen. Live percent was successfully estimated in 1% eosin stain. Sperm abnormality was also fairly identifiable in the same slide prepared for determining sperm viability. The most common sperm defect was bent tail (40.9± 2.9%; $p<0.05$) as compared to head (17.6±01%) and other sperm defects. Sperm longevity declined with time and total motility was reduced to <60% at 2 h post ejaculation at 37°C water bath temperature. However, after 24 h of chilling, total motility remained at 36.2±4.3% while progressive motility was 24 ±7.4%.

Pregnancy rate was 40% (2/5) in *Sennar* jennies, 58.3% (7/12) in Abyssinian jennies and 64.7% (11/17) in mares. Pregnancy confirmation at early stage using ultrasonography was fairly easier in all cases (Figure 2).

DISCUSSION

Although donkey population in Ethiopia is high population of *Sennar* donkeys is very low. Sparsely located within the country, they are mostly used in the production of best mule using hand mating. Their semen characteristics have never been assessed. Artificial insemination with cooled transported semen would allow the development of appropriate breeding plans and a

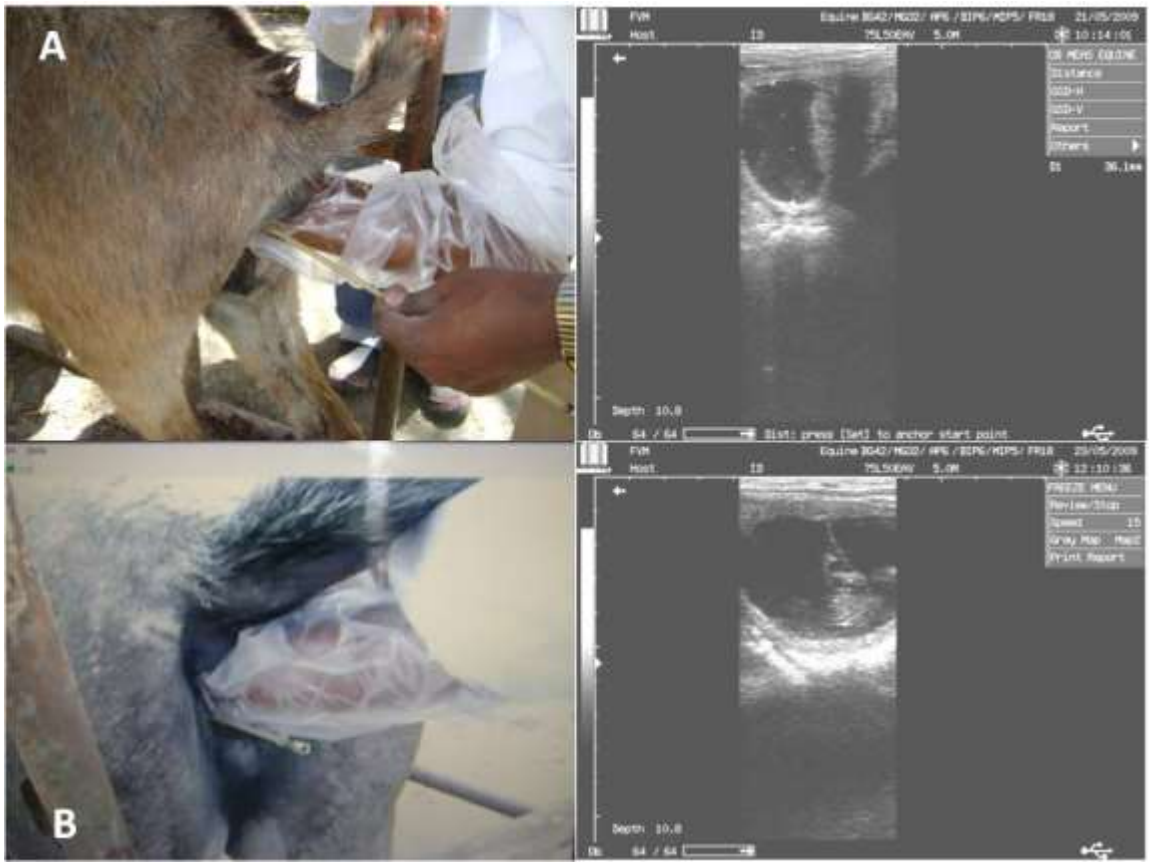

Figure 2. AI in Abyssinian donkey and 30-day embryo of the same jenny, AI in mare and 35-day embryo of the same mare.

better gene distribution, reducing the risks of excessive inbreeding in small populations (Canisso et al., 2011). Semen characteristics are very good showing comparatively better sperm motility; however this quickly deteriorates through time and particularly after chilling. Total volume and concentration are lower than Amiata breeds of donkeys (Rota et al., 2008), and are higher than Abyssinian donkeys (Lemma and Deressa, 2009) but comparable to report by Purdy (2005). Apart from semen characteristics, an important difference from stallion might be the continued reluctance of jacks to mount jennets during semen collection. Tail abnormality is much higher than previous reports for other donkeys (Henry et al*.,* 1991). Pregnancy rate is affected by factors such as the freezing technique, extenders used, time of insemination, number of spermatozoa used for the AI (Rota et al., 2012; Saragusty, 2015). Previous study (Vidament et al., 2009) in donkey semen showed that pregnancy with fresh or chilled semen is similar for jennies and mares. Previous studies confirm freezing donkey semen with addition of glycerol can reduce pregnancy dramatically. An improved technique of freezing large volumes of semen as in directional freezing has been found to improve quality of frozen semen and fertility (Arav and Saragusty, 2013). Some studies support the addition of homologous seminal plasma during re-suspension of frozen semen to improve fertility (Okazaki et al., 2012; Sabatiniet al., 2014). If seminal plasma has influenced the pregnancy rate in this study has to yet be verified. Pregnancy rate however is still much better than the 40% previously reported by Oliveira et al*.* (2006).

Conclusion

Evaluation of semen characteristic as part of the breeding soundness evaluation can give a more objective assessment of *Sennar* jacks breeding ability. Outcomes of semen analysis in the present study are generally good with acceptable level of fertility both in jennies and mares. The application of multiparametric evaluation could further improve quality of semen that can be used for AI. A notable setback observed in this study is the reluctance of the jacks to mount even on jennies well into estrous. AI both in mares and jennies also require meticulous ultrasonic evaluation of ovulatory follicle to match the time of ovulation with the time of insemination to get good pregnancy results.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Full Length Research Paper

Sperm storage capacity and total protein concentration in the testes of bucks in the native tropical environment

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Comparison was made for sperm storage capacity and total protein concentration in the testes of postpubertal West African Dwarf (WAD) and Sokoto Red (SR) bucks, this was determined using 12 postpubertal bucks. The average weight of the breeds was 7.50 ± 0.35 kg for the WAD and 11.00 ± 0.71 kg for the SR bucks. All the animals were slaughtered and their testes excised. Portions of the reproductive tracts were homogenised and biochemical assessment was carried out using standard procedure and data obtained were subjected to t-test. While gonadal sperm reserves were similar ($p>0.05$) between the breeds, all extragonadal regions of the reproductive tract differed significantly ($p<0.05$) between the breeds. With respect to total protein, significant differences ($p<0.05$) were recorded in testis, corpus epididymis and ductus deferens with clear similarities ($p>0.05$) in the caput and cauda epididymal regions for both breeds. Meanwhile, very high and positive relationship were also observe between caudal sperm motility and corpus epididymis with the values, r= 0.84; ***$p<0.001$ and r= 0.78; ***$p<0.001$ in the WAD and SR bucks, respectively. The relationship in the testis and the caudal epididymis for both breeds were high and positively correlate with values, r= 0.73; **$p<0.01$ in the WAD and r=0.71; **$p<0.01$ in the SR bucks. The significantly high extragonadal sperm reserves and protein activity in the testes of this breeds confirmed its good attribute for a selection programme in the native tropical environment.

Key words: West African Dwarf, Sokoto Red, testes, genotype, bucks.

INTRODUCTION

The system of goat production in Nigeria is basically traditional with animals are left to scavenge for feed, while commercial production is in the hands of a few farmers whose production has not met up with the rising demand for animal protein in our native environment. However, goats have considerable advantage over other

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classes of livestock in Nigeria. Besides its ability to thrive under extreme conditions of natural location and its flexible integration into the most diverse socioeconomic conditions, it is small in size and commonly reared among women and children, especially in the rural areas (Ngere, 1983; Gallaha and Pitman, 2001). Apart from milk and dung, goat meat in form of 'isi-ewu' is used to garnish delicacies in the eastern part of Nigeria and it is an animal recommended for sacrificial offerings in the annual festival of thanksgiving by the Royal Benin Kingdom of South-South Nigeria. The West African dwarf goats are mostly common in Nigeria; it is of a superior and most popular genotype and it has for long been known to be more prolific owing to the number of kid born per doe per year (Gall et al., 1992). The Sokoto red goats on the other hand is of great economic importance in that, the popularity of the high "Moroccan Leather" has been of great benefit to Europe since the medieval period (Bitto and Osayande, 2013). The study on the epididymal sperm reserve of goats came to light when Corteel (1973) reported on goats of 140 and 168 days of age. Jindal and Panda (1980) gave similar report on epididymal sperm reserves in adult billies a decade after. In the ram, there are wide variations in the extragonadal sperm reserves (ESR) as reported by different authors. Researchers have also reported values of 27.13 x 10^9 (Dot and Skinner, 1967) from same breed. The correlation between testicular weight and epididymal sperm numbers as reported by Ugwuegbu et al. (1985) in the Maradi buck found that mean epididymal sperm was highly and positively correlated with testicular sperm count. Bitto (1989) reported that extragonadal sperm reserves for the pubertal WAD bucks were similarly unaffected by season. However in the early 1990s and the millennium, several authors have reported on the gonadal and extragonadal sperm reserves in the pubertal WAD buck (Inegedu et al., 2005), adult Sokoto red bucks (Bitto et al., 2008). Information on the WAD and Maradi buck direct comparison is lacking, which leaves a lot of question on the sexual activity that takes place at the post pubertal phases in these two breeds with economic importance in our native environment. Evaluation of biochemical characteristics enhances the assessment of the functional state of the epididymis in which the spermatozoa are stored awaiting ejaculation during natural mating or artificial insemination. Malfunction of both epididymis and other assessor glands affect the fertilizing capacity of the spermatozoa adversely (White, 1973). More recently, Ewuola et al. (2014) reported a significant decline in the total protein concentration in the testis of WAD bucks fed varied levels of aflatoxin, although, the improvement and commercial production of the West African Dwarf and Sokoto Red goats is still scanty at the post pubertal stage and there is still a dearth of knowledge on the reproductive physiology on these breeds of goats. However, this work was carried out to evaluate the gonadal, extragonadal sperm reserves and protein concentrations in the testes of post pubertal WAD and RS bucks in their native environment.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in the Animal Physiology and Bioclimatology Laboratory of the Teaching and Research Farm, University of Ibadan, South West, Nigeria. Ibadan is on Latitude 7 20' N and longitude 3 50' E.

Animals and management

Twelve post pubertal bucks of 7 to 8 months, with an average weight of 7.5± 0.61 kg for the WAD and 11.00±1.22 kg for the RS bucks were utilized for this study. The animals were housed in two separate pens and diets supplemented with composite dried cassava peels were fed to meet the nutrient requirement of goats. Animals were allowed to freely graze on forage and ample drinking water was provided *ad libitum*.

Data collection and statistical analysis

At eight weeks of observatory feeding, animals were all sacrificed by restraint and exsanguination; the reproductive tracts were decapitated and frozen at -20°C. The testes, epididymis and the ductus deferens was carefully separated, trimmed off adhering fat and weighed individually. The epididymis was divided into the caput, corpus and cauda regions on the basis of external morphology. Estimate of the testicular and epididymal sperm reserves was determined by the homogenization technique of Amann and Almquist (1962) and Amann (1970) and as also reported by Egbunike (1980). Five grams weight of the testes, epididymis (caput, corpus and cauda) and ductus deferens was homogenized in 100 ml of 0.154 m NaCl in clean beakers with a pair of scissors for 5 min after which each of the homogenates was filtered through two layers of loosely netted bandage into clean glass test tubes. The protein concentration of testicular spermatozoa from the testis, caput, corpus and caudal epididymis and ductus deferens were evaluated using the biuret method of Weichselbaum (1946). Daily sperm production (DSP) was determined by dividing the values obtained from the testicular homogenates by a time divisor 3.56. The DSP/g were evaluated by dividing the corresponding DSP value by weight of testicular tissue obtained (Amann, 1970). Data obtained was subjected to analysis of variance ANOVA, using t-test between the regions of the testes of both breeds (SAS, 2013).

RESULTS

The comparative differences in paired testis weight (PTW), daily sperm production (DSP) and daily sperm production per gram testis (DSP/g) is as indicated in Table 1. DSP did not differ significantly ($p>0.05$) with values 0.48±0.05 (x10^8) g/100 ml and 0.48±0.05^a g/100 ml for the WAD and SR bucks, respectively. Although, PTW and DSP/g testis showed significance ($p<0.05$) with

Table 1. Daily sperm production (DSP) and daily sperm production per gram (DSP/g) testis rate in postpubertal WAD and SR bucks.

Variables	WAD	RS	Significant Level
Live weight (kg)	7.50±0.61[b]	11.00±1.22[a]	p<0.05
PTW (g/100 ml)	6.83±0.51[b]	7.53±0.45[a]	p>0.05
DSP (x10^7)g/100 ml	0.48±0.05	0.48±0.02	p>0.05
DSP/g (x10^7)g/100 ml	0.21±0.04	0.18±0.03	p>0.05

P<0.05 = Significant, p>0.05 = not significant, SEM (±) = standard error mean, WAD = West African dwarf bucks, SR = Sokoto red bucks, SL = significant level.

Table 2. Differences between post pubertal WAD and Sokoto Red bucks in sperm storage capacity (mean ± SEM).

Region of the reproductive tract	WAD	SR	Level of significance
Testis reserves (10^8)	1.71 ± 0.18[a]	1.70 ± 0.08[a]	p>0.05
Contribution of gonads (%)	29.80	31.05	
Caput reserves (10^8)	0.49 ± 0.06[b]	0.58 ± 0.03[a]	p<0.05
Contribution of caput (%)	8.51	10.61	
Corpus reserves (10^8)	0.26 ± 0.03[b]	0.29 ± 0.04[a]	p<0.05
Contribution of corpus (%)	4.55	5.30	
Cauda reserves (10^8)	3.06 ± 0.26[a]	2.69 ± 0.29[b]	p<0.05
Contribution of cauda (%)	53.31	49.20	
Ductus reserves (10^8)	0.22 ± 0.09[a]	0.21 ± 0.08[a]	p>0.05
Contribution of ductus (%)	3.83	3.84	
Total epididymal reserves (10^8)	3.81 ± 0.35[a]	3.56 ± 0.36[b]	p<0.05

SEM = Standard error of mean, p<0.05 = significant, p>0.05 = not significant, a, b = values bearing different. Superscripts on the same row are significantly different, WAD = West African dwarf bucks, SR = Sokoto Red.

values, 6.73±0.39 kg g/100 ml to 7.63±0.05 kg and 0.21±0.04(×10^8) g/100 ml to 0.18±0.03(×10^8) g/100 ml for the post pubertal WAD and SR, respectively. Differences between the post pubertal WAD and SR bucks in sperm storage capacity as indicated in Table 2 showed similarities (p>0.05) in the testis region with values of 1.71±0.18(×10^8) g/100 ml in the WAD and 1.71±0.15(×10^8) g/100 ml in the SR bucks, with percentage contributions of 29.80 and 31.05%, respectively. Estimate of the extragonadal sperm reserves ranged from the caput to the cauda epididymis with values of 0.49±0.07(x10^8) g/100 ml to 3.06±0.26(x10^8) g/100 ml in the WAD and 0.38±0.03(x10^8) g/100 ml to 2.68±0.29(x10^8) g/100 ml in the SR bucks. However, significant differences (p<0.05) were observed for the caput, corpus and cauda epididymis with clear similarities (p>0.05) in the ductus regions for both breeds. The cauda regions recorded the highest level of contribution with the values of 53.31 and 49.20% in the WAD and SR bucks, respectively. Total protein concentrations in the reproductive tract in both breeds as indicated in Table 3, followed the order: testis, corpus epididymis, cauda epididymis, caput epididymis and ductus deferens in the WAD and ductus deferens, cauda epididymis, testis, caput epididymis and corpus epididymis in the SR bucks. There were significant differences (p<0.05) in the testis, corpus epididymis and ductus deferens, while the cauda and caput regions did not differ significantly (p>0.05). Estimated values ranged from 0.41±0.15 g/100 ml in the ductus deferens and 1.81±0.85 g/100 ml in the testis in the WAD, while the SR bucks had values ranging from 0.31±0.25 g/100 ml in the corpus epididymis to 1.05±0.24 g/100 ml in the ductus deferential region. Protein concentration in the reproductive tract, both breeds exhibited significant differences (p<0.05) in the testis, corpus epididymis and cauda epididymis with values ranging from 0.41±0.15 to 1.81±0.85 in the WAD and 0.31±0.25 to 1.05±0.24 in the SR bucks. Table 4 shows the correlation matrix between cauda epididymal sperm motility, mass activity and protein concentrations in sperm cells and fluids in post pubertal WAD and SR bucks. Both breeds displayed a high and positive correlation between the testis (r= 0.97; ***p<0.001) and corpus epididymis (r= 0.97; ***p<0.001). A very high and positive correlation were also observed between caudal sperm motility and corpus epididymis

Table 3. Protein concentrations in sperm cells in post pubertal WAD and SR bucks.

Regions of the reproductive tract	WAD (g/100 ml)	SR(g/100 ml)	Level of significance
Testis	1.88±0.85[a]	0.84±0.14[b]	p<0.05
Caput	0.78±0.14[a]	0.49±0.29[b]	p<0.05
Corpus	1.05±0.19[a]	0.38±0.23[b]	p<0.05
Cauda	0.75±0.16[a]	0.94±0.34[a]	p>0.05
Ductus	0.48±0.11[b]	1.05±0.24[a]	p<0.05

p<0.05 = significant, p>0.05 = not significant, SEM = standard error mean, a, b = values bearing different superscripts in the same row are significantly different, LOS = level of significance.

Table 4. The correlation matrix between cauda epididymal sperm motility, mass activity and protein concentrations in sperm cells and fluids in the post pubertal WAD and SR bucks.

S/N	7	6	5	4	3	2	1
1	0.36	0.76**	0.84**	0.83**	0.57*	-0.58*	-
2	0.93***	0.55*	-0.36	0.52*	-0.53*	-	0.88**
3	0.49	0.73**	0.97***	0.95***	-	0.14	0.36
4	0.58	0.97***	0.69*	-	0.47	0.46	0.57*
5	0.33	0.73**	-	0.44	0.91***	0.35	0.45
6	-0.46	-	0.79**	0.41	0.97***	0.82**	0.66*
7	-	1.00***	0.78**	0.41	0.97***	0.83**	0.65*

*= p<0.05, **=p<0.01, ***p=0.001, 1. Cauda sperm motility, 2. mass activity, 3. testis, 4. caput, 5. corpus, 6. cauda, 7 ductus deferens.

with the values (r= 0.84; ***p<0.001 and r= 0.78; ***p<0.001) in the WAD and SR bucks, respectively. Meanwhile, the WAD bucks displayed a low and negative correlation between mass activity and corpus epididymis (r= -0.36; p<0.05), with low and positive correlation (r= 0.46; p<0.05) between caudal epididymal sperm motility and caudal epididymis in the SR bucks. Comparing the relationship in the testis and the caudal epididymis, both breeds displayed a high and positive correlation with values r= 0.73; **p<0.01 in the WAD and r=0.71; **p<0.01 in the SR bucks.

DISCUSSION

The relative contributions of gonadal and epididymal sections in the present study (at the post pubertal phase) followed a similar pattern. The values of sperm reserves obtained are generally lower than values earlier reported in pubertal WAD bucks, values were in line with earlier reports of Inegedu et al. (2005) in the WAD bucks and Carew and Egbunike (1980), Ugwuegbu et al. (1985) in the RS buck. The higher sperm reserves in the cauda epididymal regions confirm well established fact that the cauda epididymis is the store house of spermatozoa in several animal species. The higher sperm storage capacity in the post pubertal WAD and RS bucks confirms earlier reports of Oyeyemi and Ubiogoro (2005) in large boars and Sharma and Gupta (1978) in buffalo bulls. This could have taken into account sperm transit time which is determined by number of factors including frequency of ejaculation and the demand placed on testicular spermatozoa post collection. Gonadal sperm reserve values were also lower for that obtained by Ewuola et al. (2014) and this disparity could be as a result of factors due to nutrition, age and genetics in this study. The values obtained in protein concentrations in the testes of both breeds fell within the range, although values were lower than that obtained more recently (Ewuola et al., 2014) in WAD bucks. The significant differences (p<0.05) observed for both genotypes in the caput and corpus epididymis could be due to breed effect, in that adjustment occur during the ripening of spermatozoa in order to cater for buffering capacity (Wildeus and Enwistle, 1982; Weisgold and Almquist, 1979); so far no documented report on the protein concentrations in these regions in the SR bucks. Protein levels in the testis were highest in the WAD with the SR bucks recording the highest in the ductus differential regions. The significant differences (p<0.05) observed for both breeds in the corpus epididymis could be due to breed effect (Wildeus and Enwistle, 1982; Weisgold and

Almquist, 1979). Report on the protein concentration in these regions in the SR buck has not been documented. It is worthy to note that the values obtained in the post pubertal WAD were in the order: testis, corpus epididymis, cauda epididymis, caput epididymis and ductus deferens and that of the SR bucks were also in the order: ductus deferens, cauda epididymis, testis, caput, corpus epididymis. The relationship between cauda epididymal sperm motility, mass activity and protein concentrations in sperm cells and fluids in post pubertal WAD and SR bucks account for the fact that sperm cells undergo ripening to aid buffering process (Egbunike et al., 1985; Butswat and Zaharadeen, 1998).

Conclusion

Even though the age of the animals used in this study is just after puberty, the relationship between activity in the testis and the sperm store house were normal. Therefore, data generated from this study has highlighted some attributes in relationship between *in situ* sperm production and sperm reserves in the testes. It is concluded from these results, that even though bucks of these breeds may be relatively low in sperm storage capacity at the post pubertal phase, they could be used cautiously as sires and genetic resources for conservation and improvement.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Review

Bacterial pathogens of pigs with particular reference to *Escherichia coli*: A systematic review and meta-analysis

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Pigs are ungulate animals of the genus *Suis* and family Suidae. They are globally spread but restricted in certain countries due to religious and cultural beliefs. Pork serves as an important source of protein (38% of meat consumed in the world). While pig production remains a profitable enterprise, commercial and particularly the small-scale farmers face huge constraint in this husbandry practice, one of the most important being bacterial infections and its associated with morbidity and mortality. In this work, we reviewed the prevalence of bacterial infections in pigs with particular reference to *Escherichia coli*, a bacterium that is regularly isolated and can lead to multiple infections in pigs. Literatures were searched on selected veterinary and biological data bases in 2016 with focus on natural infections and isolates from natural infections with epidemiological details. Pathotypes, serotypes and serogroups of *E. coli*, the country of origin, source, growth stage, age of pigs infected, disease outbreak, the number of samples and type of samples, numbers and percentage of positive samples and isolates were used as filters. Pathotypes reported include enterotoxigenic *E. coli* (ETEC) 66.7%, enterotoxigenic *E. coli* and shiga toxigenic *E. coli* (ETEC and STEC) 14.3%, STEC only (7.9%), enterotoxigenic *E. coli*/enteropathogenic *E. coli*/enteroaggregative *E. coli* (ETEC/EPEC/EAE) 31.7%. Others were enterohaemorrhagic *E. coli* (EHEC), diffusely adherent *E. coli* (DAEC) (ETEC, EPEC, STEC) and extra-intestinal pathogenic *E. coli* (ExPEC). Twenty-nine countries with documented records of cases of *E. coli* were included with the USA reporting, the highest number followed by China. About 74% of the samples were taken from farms and others were from samples submitted to research laboratories and veterinary faculties for necropsy. Serogroups O141, O149, O139, O138, O8 and O9 were most common. Piglets were most affected (52.3%) followed by weaners (39.6%) and porkers (7.9%) with age ranging from 1 to 392 days old. A total of 24,854 isolates were considered, 10477 (42.2%) were positives and the following genes were carried: STa, STb, LT, stx1, stx2, Stx-2e, F4, F5, F6, F18, F41, AIDA, EAST1, eae, paa and hlyA. The diseases produced by *E. coli* were neonatal diarrhoea, colibacillosis, post-weaning diarrhoea and edema disease. The associated risk factors were poor housing, management and feed changes, extensive use of antibiotics as prophylaxis, overcrowding, and high humidity and temperature changes. India, USA, Japan, Slovakia, Denmark Sweden and Poland were countries with significant reports and high detection of virulence factors (72 to 100%).

Key words: *Escherichia coli,* diarrhea, serogroups, enterotoxigenic, colibacillosis.

INTRODUCTION

Pigs are ungulate animals of the genus *Suis* and family Suidae. Domesticated pigs originated from the European wild boar *Susscrofa* are indigenous to the Eurasian and African continents (Giuffra et al., 2000).The global pig population is estimated to be approximately one billion (Statista, 2016), and although this spread across the world, it may be restricted in certain countries due to religious and cultural beliefs. Pork serves as an important source of protein (38% of meat consumed in the world) and as a means of livelihood especially for women in developing countries (Madzimure et al., 2012). Pigs are also kept for leather, hair, as pets and use in human research (Gosh, 2014).

While pig production remains a profitable enterprise, commercial and particularly the small-scale farmers face huge constraints in this husbandry practice, one of the most important being bacterial infections and its associated with morbidity and mortality. We reviewed the prevalence of bacterial infections in pigs and paid particular attention to *Escherichia coli*, a bacterium that is regularly isolated and can lead to multiple infections in pigs.

The key words used to gather literature for review include; " *Escherichia coli* or *E.coli*", "pig, swine or porcine", "outbreak", "diarrhoea", "Oedema disease", "post weaning diarrhoea", "colibacillosis" and "prevalence". Literature searches were performed on selected veterinary and biological databases including the CAB Abstract, Medline, Pubmed, Science Direct and Google Scholar between January and November, 2016. Particular consideration was given to natural infections and isolates from natural infections with epidemiological details. Pathotypes, serotypes and serogroups of *E. coli*, the country of origin, source, growth stage, age of pigs infected, disease outbreak, the number of samples and type of samples, numbers and percentage of positive samples and isolates were used as filters. All literature considered were in English or where available then the English translations were used.

Extracted and compiled published manuscripts from peer reviewed journals were quality-checked and duplicate documents were removed. All remaining documents (n = 61) were filtered, harmonized and coded in a single Microsoft Excel® spreadsheet. The number of events, sample sizes and outcomes were calculated based on the available data. All data was analysed using the Fixed-effect model (precision-based estimates) in the Meta-analyses software on Excel (Neyellof et al., 2012). Comparison between individual studies was calculated in WinPepi v11.24 (Abramson, 2011) and presented in percentages with 95% confidence intervals. Cumulative events with measures of central tendencies were also produced in forest plots.

Bacterial pathogens of pigs

Based on our evaluation, the bacteria that affect pigs are diverse and vast but are not limited to the following, grouped by areas of primary lesions:

Cutaneous (skin) associated bacteria

Staphylococcus species

Infection in pigs is caused by *Staphylococcus hyicus* resulting in exudative epidermitis (Greasy pig disease) (Andresen, 1998). *Staphylococcus hyicus* is composed of both non-virulent and virulent strains which produces an exfoliative toxin, responsible for skin alteration in exudative epidermitis of pigs (Wegener et al., 1993; Andresen et al., 1997).

It is characterized by sudden onset of excess sebaceous secretion and exudation from the skin without pruritus leading to dehydration, growth depression and possibly death (Taylor, 2013). Other Staphylococcus species that could be isolated are *Staphylococcus chromogenes* and *Staphylococcus Sciuri* (Chen et al., 2007). *Staphylococcus aureus* is another important and common pathogen isolated from swollen ears, umbilical abscesses, subcutaneous abscesses and foot lesions, (Taylor, 2013; De Neeling et al., 2007).

Treponema species

Treponema species are associated with skin or mucous membrane diseases and cause skin ulcers in pigs regularly (Karlsson, 2014). Three major phylotypes are involved in infections which include: Treponema pedis (the most predominant), Treponema parvum and an undesignated phylotype (Karlsson, 2014).

Treponema pedis infection can occur as cutaneous spirochaetosis, ear necrosis and spirochaetal granuloma. They have been indicated as secondary bacterial infection in severe and chronic skin lesions of pigs (Taylor, 2013; Karlsson, 2014), such as ulcerative porcine stomatitis (Jensen et al., 2014) and periodic outbreaks of ear necrosis among weaners and gingival infections (Pringle et al., 2009; Karlsson et al., 2013).

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Reproductive system associated bacteria

Leptospira species

Leptospirosis, caused by *Leptospira* species is a disease that occurs worldwide in pigs and infection which is more common in animals kept outdoors (Ryley and Simmons, 1954). Route of infection is by ingestion, direct contact and through abrasions, trans-placental transmission or the veneral route (Taylor, 2013). *Leptospira* species are fine, spiral, aerobic, motile, gram negative bacteria (spirochete) of about 10 µm in length and 0.2 µm in diameter (Faine, 1994).

Approximately, 13 serovars of *Leptospira* are involved in infections in pigs and associated primarily with reproductive losses in breeding herds. The organisms persist in the kidneys and genital tracts of carrier pigs and are excreted in urine and genital fluids (Taylor, 2013). Routine vaccination may reduce the effect of the bacteria in a herd (Whyte et al., 1982), but elimination may be a difficult target because, pig is a reservoir host for leptospirosis.

Brucella species

Brucella suis infection in pigs, occurs mainly through the venereal or oral route (Xavier et al., 2010). Infection develops as bacteremia may persist for as long as 90 days which may lead to localisation in various tissues with resultant stillbirths, abortions, orchitis, lameness, posterior paralysis, spondylitis, occasional metritis and abscess formation. Infertility may occur in both sexes (Xavier et al., 2010; Deyoe, 1967).

Other pathogenic species in pigs are *B. abortus* and *B. mellitensis* (Godfroid, 2002). Diagnosis is mainly through the brucellosis card (Rose Bengal) test; but serum agglutination tests or complement fixation tests have also been used (Nicoletti, 2010). Brucellosis in pigs has a global distribution however its prevalence in domestic pigs is low in some countries and known to have been eradicated in USA (Godfroid, 2002).

Listeria species

Infection of pigs with *Listeria* species is common and previous study in Japan, Denmark and Yugoslavia suggested that approximately 10% of pigs at slaughter are infected (Taylor, 2013; Lungu et al., 2010). *Listeria monocytogenes* infection rarely causes disease, but sudden death in piglets, septicaemia and nervous signs have been recorded.

Most authors concurred that, porcine listeriosis occurs mainly as a septicaemia in piglets less than ten days old (Ladds et al., 1974; Long and Dukes, 1972; Busch et al., 1971) and multifocal hepatic necrosis is often the most notable necropsy finding in piglets (Lopez and Bildfell, 1989). Abortions, stillbirths and birth of weak piglets may also occur in sows (Taylor, 2013; Vannier, 1999). *Listeria* infection and carriage in domestic pigs or wild boar is a potential source of infection for man (Taylor, 2013; Borch et al., 1996).

Erysipelothrix species

Erysipelothrix rhusiopathiae is a small, facultative anaerobic, Gram positive (G+ve) rod that causes a condition known as swine erysipelas (Opriessnig et al., 2010; Wood and Steele, 1994). The disease outbreak may present as acute, sub-acute or chronic. It is characterized by sudden death or fever associated with characteristic diamond skin lesions (Opriessnig et al., 2010). Arthritis, vegetative endocarditis and abortion in pregnant sows may be observed (Schrauwen et al., 1993).

Necropsy lesions include enlarged and congested lymph nodes, oedematous and congested lungs, splenomegaly, hepatomegaly, petechial haemorrhages on the kidneys and heart. *E. rhusiopathiae* causes considerable economic losses and remains an animal hygiene problem in swine production areas of the world (Takeshi et al., 1999), and up to 50% of pigs in the world are estimated to harbour the organism in their tonsils and lymphoid organs (Opriessnig et al., 2010).

This status results in shedding of the organism in urine, faeces, saliva and nasal secretions (Opriessnig et al., 2004).

Respiratory system associated bacteria

Actinobacillus species

Actinobacillus suis and *Actinobacillus equuli* are two important species that may cause fatal septicaemia, endocarditis and arthritis in pigs of 1 to 6 weeks of age. In older animals, skin lesions and focal necrotising pneumonia, valvular endocarditis, abortion, metritis and polyarthritis may be seen (Radostits et al., 2000; Ramos-Vara et al., 2008). Clinical signs and post-mortem lesions are not specific but suggestive of the disease (Taylor, 2013).

Additionally, *Actinobacillus pleuropneumonia* may cause a respiratory infection of weaned, growing and finishing pigs in which there is fibrinous pleurisy and pneumonia with characteristic infarcts in the lungs. Infection which is highly contagious, often fatal and progressive weight loss in chronically affected pigs may be observed (Taylor, 2013; Frank et al., 1992).

Mycoplasma species

Mycoplasma organisms have been isolated from pigs but

only four species have repeatedly been associated with clinical disease namely (1) *Mycoplasma hyorhinis* which cause polyserositis and arthritis in young pigs of about 3 to 10 weeks; (2) *M. hyosynoviae* causes arthritis in growing pigs weighing between 35kg and 110kg live weight (Taylor, 2013); (3) *Mycoplasma hyopneumoniae* which is a primary pathogen of enzootic pneumonia, a chronic respiratory disease in pigs, highly prevalent in almost all pig producing areas. It is also considered to be one of the primary agents involved in the porcine respiratory disease complex (PRDC) (Thacker, 2006).

The organism is primarily found on the mucosal surface of the trachea, bronchi and bronchioles (Blanchard et al., 1992); and (4) *Mycoplasma suis* (formerly *Eperythrozoon suis*) infection which affects piglets 0 to 5 days of age, but weaners, growers and sowers may also be infected. Route of infection is by parenteral, transplacental or oral transmission and the clinical disease may manifests in 5 to 6 days.

The acute phase involves fever, anaemia, icterus, unthriftiness, jaundice and poor growth in weaned pigs, and is associated with low morbidity at high mortality rates (Messick, 2004). The chronic phase results in low reproductive efficiency, growth retardation, abortions, stillbirths and agalactia in sows (Messick, 2004; Heinritzi, 1989). In addition, a study revealed that *Mycoplasma arthritidis*, a rodent *Mycoplasma* has also been isolated from joints in an outbreak of conjunctivitis, severe polyarthritis and infertility in a boar stud (Binder et al., 1990).

Bordetella species

*Bordetella bronchiseptica*is regarded as the aetiologic agent of atrophic rhinitis, colonises the ciliated epithelium of the upper and lower respiratory tract at about one week of age in piglets. It causes rhinitis characterised by sneezing, shortening or twisting of the snout, hypoplasia, mild nasal turbinate atrophy, persistent purulent bronchitis, haemorrhage, pneumonia and impaired growth (Giles, 1992; Duncan et al., 1966; Mazumder et al., 2012).

Bordetella bronchiseptica predisposes pigs to colonization and disease with other bacteria such as *Pasteurella multocida* and *Haemophilus parasuis* (Brockmeier, 2004; Brockmeier et al., 2001).

Pasteurella species

Pasteurella multocida is associated with pneumonia and atrophic rhinitis in pigs and can result in important economic losses on large pig farms worldwide (Davies et al., 2003; Dziva et al., 2008). Its strains are grouped into five capsular serogroups; A, B, D, E and F , however only serogroup A,B and D have been recovered from pigs (Davies et al., 2003; Townsend et al., 1998; Tang et al., 2009). Toxigenic *P. multocida* serogroups A and D together with *Bordetella bronchiseptica,* coexist to cause atrophic rhinitis (Davies et al., 2003; Backstrom et al., 1988).

Toxigenic *P. multocida* infection results in severe sneezing in non-immune piglets. This is later followed by atrophy of the turbinate bones and a distortion of the nasal septum, shortening and twisting of the upper jaw which may be accompanied by reduction in the rate of weight gain (Taylor, 2013). Similarly, pneumonic pasteurellosis is also a condition that results from the colonisation of existing lung lesions with *P. multocida* which may give rise to fever, respiratory distress and death in some cases and it is typically associated with sub-acute or chronic pleuritis. Common route of transmission is by nose-to-nose contact; however, both vertical and horizontal transfer may occur (Taylor, 2013; Davies et al., 2004).

Mannheimia (Pasteurella) haemolytica has been isolated in piglets from localised areas of fibrinous pleurisy or pleuropneumonia and from outbreaks of diarrhoea in pigs (Taylor, 2013).

Haemophilus species

Haemophilus parasuis is found in the upper respiratory tract of pigs as a commensal bacterium, but invades and cause severe systemic disease under favourable conditions (Oliveira et al., 2003). It causes Glassers disease and acute septicaemia (Peet et al., 1983; Riley et al., 1977). Glassers disease is an infectious, sometimes fatal polyserositis, polyarthritis and meningitis of young pigs (Amano et al., 1994). Bronchitis and other syndromes may occur in older animals in non-immune herds (Taylor, 2013).

Transmission is by direct contact and all age categories of pigs are susceptible to the infection (Oliveira and Pijoan, 2002).Post mortem lesions include serofibrinous or fibrino-purulent exudate on mucosal surfaces, usually in peritoneum, pericardium, pleura or joint surface. In the septicaemic form, petechial and ecchymotic haemorrhages are detected in liver, kidneys and brain (Amano et al., 1994).

Mycobacterium species

Several species of *Mycobacterium* such as *Mycobacterium porcinum, Mycobacterium avium* subsp. *hominisuis*, *Mycobacterium bovis*, *Mycobacterium intracellulare, Mycobacterium fortuitum* and *Mycobacterium tuberculosis* have been associated with tuberculosis in pigs. *Mycobacterium avium* subsp. *hominisuis* is an opportunistic pathogen, infecting mainly pigs and humans (Mijs et al., 2002; Inderlied et al., 1993; Thorel et al.,

2001).

A recent study demonstrated cross-reactions between avian and bovine tuberculin in pigs (Agdestein et al., 2011). *Mycobacteria bovis* is the main agent causing tuberculosis in cattle, while *M. tuberculosis* primarily causes tuberculosis in humans.

However, they both belong to the *M. tuberculosis* complex (MTC) and can lead to infections in pigs (Komijn et al., 1999; Biet et al., 2005). Tuberculosis is mostly observed in pigs at slaughter when gross lesions are detected primarily through the examination of the lymph nodes of the head and the visceral regions and partial or full carcasses condemnation usually follow, because it is a potential risk to human health.

Streptococcus species

Streptococcus suis is an encapsulated gram positive coccus and occurs singly in pairs, or occasionally in short chains. It is a normal flora in the upper respiratory tract of pigs, the genital and digestive tracts (Higgins and Gottschalk, 1999). *S. suis* causes septicaemia, arthritis and meningitis in suckling piglets and post-weaning pigs but less commonly in finishing pigs (Taylor, 2013; Gottschalk et al., 2007). The organism is an emerging zoonotic agent responsible for septicaemia which may sometimes be accompanied by septic shock and meningitis in humans (Goyette-Desjardins et al., 2014).

Streptococcus porcinus infection also occurs in pigs and is sometimes referred to as streptococcal lymphadenitis or streptococcal abcess. It causes abscess particularly in the cervical lymph nodes (Taylor, 2013). The Lancefield group C and β-haemolytic streptococci are other streptococci infections in pigs that are commonly isolated from the upper respiratory tract, pharynx, retropharyngeal lymph nodes and genital tract of carrier pigs. They are associated with vaginitis in sows and neonatal septicaemia in newborn piglets and may be isolated from arthritis and vegetative endocarditis in older animals and froms epticaemic and pneumonic lesions in older finishing pigs (Taylor, 2013). A presumptive diagnosis of infection in pigs is usually based on clinical signs and macroscopic lesions (Staats et al., 1997).

Digestive system associated bacteria

Clostridium species

Clostridial pathogens involved in pig infections include C***lostridium*** *perfringens* (type A, C), *Clostridium defficiletyphocolitis, Clostridium tetani, Clostridium novyi, Clostridium botulinum, Clostridium septicum* and *Clostridium chauvoei* (Taylor, 2013; Baker et al., 2010). *Clostridium perfringens* type C is a large gram positive rod, which occasionally forms spores, bears attachment site and produces very potent toxins. The major toxin produced is protease/trypsin-sensitive β toxin which causes fatal necrotic and haemorrhagic enteritis in piglets less than seven days old and may cause chronic infection in older piglets. Clinical signs include profuse, bloody diarrhoea, loss of weight, palour and death within 12 to 24 h (Taylor, 2013; Songer and Meer, 1996).

C. perfringens Type A causes a similar syndrome but less severe with the major toxin produced being the α-toxin (Taylor, 2013; Songer and Uzal, 2005). *C. difficile* is toxigenic and produce two major toxins, A and B (Taylor, 2013; Diab et al., 2016). It is a recognized cause of antibiotic-associated diarrhoea and pseudomembranous colitis in humans, domestic and laboratory animals (Songer et al., 2000). Infection in pigs (neonatal enteritis) can be asymptomatic or result in diarrhoea and weight loss which may be chronic in suckling pigs (Taylor, 2013). Other clinical signs such as dyspnoea, mild abdominal distension, and scrotal oedema may be observed with characteristic ulcerative lesions present in the colon at post-mortem (Taylor, 2013).

C. tetani causes tetanus which presents as stiffness and abnormal gait leading to spasm and death. The condition occurs sporadically in young pigs and may be associated with umbilical infections, castration, or ovario hysterectomy (Taylor, 2013; Meseko and Oluwayelu, 2012). Additionally, *C. novyi* type B causes sudden death in large fattening pigs and sows. Incidence is worldwide and sporadic particularly in swill-fed pigs and older sows (Duran and Walton, 1997). Food-borne botulism is caused by *C. botulinum* through a preformed toxin of this organism in food resulting in a rare, sometimes fatal flaccid paralysis in pigs. The incidence is worldwide but rarely described (Taylor, 2013; Beiers and Simmons, 1967).

Salmonella species

Salmonellosis in pigs is caused by *Salmonella Enterica* serovar Choleraesuis var kunzendorf (Salmon and Smith, 1886; Stevens and Gray, 2013; Pedersen et al., 2015). It is a host specific, facultative, intracellular pathogen that causes paratyphoid (Gray et al., 1996).

The infection may result in enteric and fatal systemic disease, however, infected pigs may carry the organism in the tonsils, intestines and the gut-associated lymphoid tissue asymptomatically (Fedorka-Cray et al., 2000; Alban et al., 2012). Transmission is primarily through the faeco-oral route (Stevens and Gray, 2013), some studies have shown that the upper and lower respiratory tract may also serve as routes of infection (Fedorka-Cray et al., 1995).The infections may be present in different forms including (1) septicaemic form which is commonest in piglets with up to 100% mortality, (2) the acute enteric form in younger and weaned pigs,(3) the chronic enteric form and (4) the diarrheic form, which is usually due to

the less invasive serotypes such as *Salmonella Typhimurium* (Taylor, 2013).

Brachyspira species

Brachyspira hyodysenteriae is a large anaerobic spirochaete that causes dysentery-infectious mucohaemorrhagic colitis of pigs (Wills, 2000). It affects pigs during the growth and finishing periods, and is characterized clinically by loss of condition with diarrhoea containing varying amounts of mucus, blood and necrotic material (Burrough, 2016).

The bacterium multiplies in the large intestine result in superficial mucosa degeneration, inflammation and multifocal points of bleeding along the mucosa. The organism does not infiltrate beyond the intestinal mucosa and results in decreased reabsorption of endogenous secretions from the unaffected small intestine leading to diarrhea (Kennedy et al., 1988). In instances, a proportion of untreated pigs may die while others may remain stunted (Taylor, 2013). Other *Brachyspira spirochaetes* that may also be involved in diarrhea are *Brachyspira innocens, Brachyspira murdochii, Brachyspira intermedia* and *Brachyspira pilosicoli* (Taylor, 2013).

Campylobacter species

Campylobacter is a gram negative, spiral, non-spore forming rod (Penner, 1988; Epps et al., 2013) and pigs are natural reservoirs with a prevalence rate of approximately 50 to 100% and excretion level of about 102 to 107 CFU/g (Jensen et al., 2006; Alter et al., 2005; Nielsen et al., 1997).*Campylobacter* infection causes a mucoid, creamy diarrhoea, which may contain blood in piglets 3 days-3weeks of age (Taylor, 2013). The species associated with disease in pigs include *Campylobacter coli* (the most common), *Campylobacter jejuni, Campylobacter hyointestinalis* and *Campylobacter sputorum* (Taylor, 2013; Alter et al., 2005*)*.

Other *Campylobacter* species present in the porcine intestine, which may multiply and become associated with enteritis, are *C. hyointestinalis subsp. hyointestinalis, C. hyointestinalis subsp. lawsonii, Campylobacter mucosalis, Campylobacter hyoilei, Campylobacter lari* and *Campylobacter lanienae* (Taylor, 2013).

Helicobacter species

This infection in pigs is caused by *Helicobacter suis*, a gram negative, spiral-shaped bacterium that is commonly found in the gastric mucosa (Hellemans et al., 2007; Grasso et al., 1996; Park et al., 2004). Piglets and porkers have highest colonisation, found in the pyloric region, however, boars and sows, also have high colonization rates in the fundic region of the gastric mucosa (Hellemans et al., 2007).

The clinical infection by this organism is rare, and the main evidence for the pathogenicity of *H. suis* was from experimental studies that showed clear association between *H. suis* infection and the development of gastritis as well as a decrease in daily weight gain (De Bruyne et al., 2012).

Lawsonia species

This organism, *Lawsonia intracellularis* is an obligate, intracellular, gram negative, small, rod-shaped, intestinal, bacterium; it is the cause of proliferative enteropathyin pigs (Guedes and Gebhart, 2003), a frequent diarrhea disease of piglets and weaners characterised by hyperplasia and inflammation of the ileum and colon (Smith and McOrist, 1997).

Study has suggested that it infect mitotically the active epithelial cells of the intestinal crypts, which later multiply and spread in the cells as they divide (Boutrup et al., 2010). The condition is often mild and self-limiting but sometimes may result in necrotic enteritis, regional ileitis and proliferative haemorrhagic enteropathy. Affected pigs appear pale, may be stunted and may die suddenly with clotted blood in the lumen of the small intestine (Taylor, 2013; Guedes and Gebhart, 2003).

Yersinia species

Yersinia species are gram negative bacilli and the species associated with pig infection include (1) *Yersinia enterocolitica,* which easily colonise the gut of neonate piglets and subsequently become healthy carriers (Skjerve et al., 1998), it is capable of causing enteritis and typhilocolitis in weaned pigs and abortion in sows (Bhaduri et al., 2005); (2) *Yersinia pseudotuberculosis* which is also carried normally as gut resident (Taylor, 2013; Laukkanen, 2010).

Acute cases are characterized by enteritis, lymphadenitis and splenomegaly while chronic cases result in granulomatous nodules and localised abscesses affecting various organs, usually the liver and lungs (Brugmann et al., 2001). About 35 to 70% of herds and 4.5 to 100% of individual pigs carry *Yersinia* species asymptomatically (Bhaduri et al., 2005).

Enterococcus species

Enterococcus durans (Lancefield Group D) is a motile gram positive cocci that has been isolated from the intestines and faeces of 3 to 5 day old piglets, usually as commensals but may sometimes be associated with diarrhea (Taylor, 2013; Cheon and Chae, 1996).

Enterotoxins and mucosal damage have been identified with diarrhoea caused by *E. durans* however, decreased activity of digestive enzymes at the mucosal brush borders have suggested that the entire pathogenesis of diarrhoea due to *E. durans* has not been completely understood (Cheon and Chae, 1996; Tzipori et al., 1984).

Bacillus species

Bacillus anthracis causes anthrax but are rare in pigs. Affected animals may die suddenly, pass bloody faeces or die after swelling of the neck. Route of entry is mainly by ingestion of contaminated feed (Taylor, 2013).

Other non-specific bacteria pathogens include

Actinobaculum species

Actinomyces suis reclassified as *Actinobaculum suis* is associated with urinary tract infections in pigs (Lawson et al., 1997; Woldemeskel et al., 2002). It is linked with cystitis-pyelonephritis complex, a syndrome in which a small group of sows or gilts pass bloody purulent urine, often soon after service. They rapidly lose condition and sudden death may supervene (Taylor, 2013).

Chlamydophila species

Chlamydophila pathogens in pigs are *Chlamydia suis, Chlamydophila pecorum, Chlamydophila psittaci* and *Chlamydophila abortus*. The infections from this organism may results in multiple lesions including conjunctivitis, enteritis, pneumonia, pleurisy, pericarditis, polyarthritis, orchitis, infertility, abortion and birth of weak piglets (Taylor, 2013; Szeredi et al., 1996; Jiang et al., 2013).

Miscellaneous bacteria pathogens isolated from pigs

(i) Since 1978, *Arcobacter* species have been associated with reproductive disorders, but excretion by clinically healthy pigs has been frequently reported as well. Information on *Arcobacter* colonization of the porcine gastrointestinal tract is lacking to date (De Smet et al., 2012).
(ii) *Aeromonas hydrophila* has been isolated from enteritis, urine infections and lymphnodes (Igbinosa et al., 2016; Gray and Stickler, 1989).
(iii) *Acinetobacter calcoaceticans, Trueperella (Arcanobacterium) pyogenes, Bacteroides fragilis*are found in the large intestine and have been isolated from diarrhoea in piglets both before and after weaning (Taylor, 2013; Hijazin et al., 2012; Myers and Shoop, 1987).
(iv) *Flavo bacterium*, a ciliated bacillus has been identified in the trachea of pigs. It has been recorded in cases of pneumonia and has been associated with lesions of active tracheitis (Nietfeld et al., 1995).
(v) *Corynebacterium pseudotuberculosis* has been recovered from the vagina and prepuce of healthy swine and from mandibular abscess of black Alentejano pigs (Kudo and Yanagawa, 1987; Oliveira et al., 2014) while *Corynebacterium ulcerance* was recovered from a case of caseous lymphadenitis in Germany (Contzen et al., 2011).
(vi) *Coxiella burneti* antibodies have been demonstrated in pigs (Taylor, 2013).
(vii) *Klebsiella* species sometimes are seen in chronic respiratory tract diseases, enteritis and mastitis (Došen et al., 2007; Ross et al., 1975; Wilcock, 1979).
(viii) *Legionella pneumophila* has been demonstrated in the sera of pneumonic pigs in the UK (Taylor, 2013).
(ix) *Burkholderia pseudomallei is* the cause of meliodosis in pigs in tropical and subtropical regions (Omar et al., 1962; Rampling, 1964).
(x) *Rhodococcus equi* usually present in granulomatous lesions in submandibular lymphnodes (Witkowski et al., 2016; Rzewuska et al., 2014).

Enteric bacteria in pigs

The pig gastrointestinal tract has a complex and dynamic microbial ecosystem, the composition of which differs between individuals, region of the gastrointestinal tract, as well as age of the animal (Konstantinov et al., 2004). This microbial flora has an important role as one of the major defence mechanisms of the animal mainly through competition for nutrients and attachment sites and stimulation of cross-reactive antibodies, which prepares the immune system in defence against pathogenic microbes (Tancrede, 1992).

The large intestine contains most of the microbial flora (over 400 species) (Sørum and Sunde, 2001) and consist of (a) strict Gram postive anaerobes and facultative anaerobes such as *Streptococci*, *Lactobacilli*, *Eubacteria*, *Bacteroides* species, *Fusobacterium* species*, Clostridium s*pecies and *Peptostreptococcus* species; and (b) facultative anaerobes such as *E.coli, Klebsiella* species*, Enterobacter* species and *Enterococcus* species (Sørum and Sunde, 2001; Jensen, 2001). Factors which can cause microbial flora changes in the pig gastrointestinal tract include psychological and behavioural stressors, environment, weaning, age, feeding systems and the pigs genotype (Burrin and Stoll, 2003).

Global pig production is most frequently and economically affected by enteric bacterial infections (Moxley and Duhamel, 1999). Common clinical signs found include diarrhoea, reduced growth rate, weight loss and death (Moxley and Duhamel, 1999). Some changes found in the intestines of pigs with enteric bacterial

infections include: attaching and effacing lesions, in enteropathogenic *E. coli* and *B.pilosicoli* infection, inflammation with *Salmonella enterica* and necrotizing and haemorrhagic lesions with certain *C. perfringens* (Moxley and Duhamel, 1999).

Lactobacilli family dominates the normal bacterial flora in pigs and produces lactic acid as an essential metabolic end-product. The concentration of lactic acid increases several-fold within the first few days post-weaning and results in decreased pH of the gut which eliminates other pathogenic enterobacteria (Janczyk et al., 2007; Pieper et al., 2008). This group of microorganisms is generally considered beneficial as their attachment to the mucosa may protect the animals from gut infection (Houdijk et al., 2002). Furthermore, cultivation-based studies have shown that lactic acid bacteria, *Enterobacteria* and *Streptococci* were the most important first colonisers of the pig intestine (Stewart, 1997). Similarly, 16S rRNA gene clone analysis indicated that ileal samples of two-day old piglets harboured a group of *E. coli, Shigella flexneri, Lactobacillus sobrius, Lactobacillus reuteri* and *Lactobacillus acidophilus* related sequences (Konstantinov et al., 2006).

E. coli

E. coli strains in pigs forms part of the normal faecal flora. However, when they acquire virulent genes they are able to cause disease (Taylor, 2013).These coliform bacteria are commonly used as representatives of the enterobacteria from faecal samples in culture based studies of the intestinal bacterial flora, as they are the major facultative anaerobic bacteria in the intestinal tract of most animal species (Dubreuil, 2012). *E. coli* are gram negative rods, flagellated with variable length and diameter of about 1µm.On culture colonies grow on solid media within 24 h after incubation and may be smooth, rough or mucoid (Fairbrother and Gyles, 2012). Major characteristics associated with pathogenic *E. coli* infections are proteins such as fimbriae and production of enterotoxins usually by the enterotoxigenic *E. coli* (ETEC) and Shiga toxin by Shigatoxigenic *E. coli* (STEC). Other toxins previously described are EAST1, cytotoxins, cytolethal distending toxin, hemolysin; outer membrane proteins (intimin) and adhesin involved in diffuse adherence (Taylor, 2013). In addition, study has shown that F18 was the main colonization factor for STEC and ETEC withF18ab and F18acas subgroups (Cheng et al., 2005).

E. coli strains have been identified as an important cause of several diseases in pigs worldwide including neonatal septicaemia, neonatal diarrhoea, post-weaning diarrhoea, oedema disease (bowel oedema or gut oedema), cystitis, septicaemia, polyserositis, coliform mastitis and urinary tract infections. They can also colonise existing lesions elsewhere in the body (Taylor, 2013; Fairbrother and Gyles, 2012). Post-weaning diarrhoea (post-weaning enteric colibacillosis) and oedema disease have a more significant impact in the porcine industry because they result in high economic losses due to high morbidity and mortality, decrease weight gain, the cost of treatments, vaccination and feed supplementation (Fairbrother and Gyles, 2012).The *E. coli* infections occur at different ages in the pigs. Coli septicaemia occurs in 0 to 4 days old piglets and may be associated with diarrhoea. Enteritis (enteric colibacillosis) which is also associated with diarrhoea, occurs at three main periods in the pigs life; neonatal diarrhoea occurs at 0 to 4 days of age, neonatal-weaning diarrhoea at 4 days to 3 to 4wks and post weaning diarrhoea usually associated with weaning, oedema disease occurs in recently weaned pigs while mastitis and cystitis occur in adult sows (Taylor, 2013).

Outbreaks of *E. coli* diarrhea have increased worldwide with post-weaning diarrhoea being the most common where F4 and F18 are usually associated with adhesion factors (Fairbrother and Gyles, 2006). This could be due to the emergence of more virulent *E. coli* clones, a benign commensal of the gut microflora which multiply rapidly and cause disease through colonisation of the intestinal mucosa or changes in the management of pigs (Fasina et al., 2015).

Furthermore, a potentially beneficial method of feeding behaviour and maintaining gastrointestinal health in pigs is through feeding weaners with liquid feed or fermented liquid feed, in contrast to dry feed, as it is considered a possible feeding strategy to maintain a high and regular feed and water intake of weaners (Canibe and Jensen, 2012). Avoiding a drastic decrease in feed and water intake after weaning is believed to ameliorate the post-weaning lag period in piglets which may predispose them to *E. coli* infections (Canibe and Jensen, 2012).

Classification of *E. coli*

The best approach to classify *E. coli* is by serotyping in association with virulent strains. However, only a small percentage of the organisms are typeable based on O, K, H and F antigens, and only about 175O, 80K, 56H and over 20F antigens have been officially recognized to date, based on proven or suspected pathogenicity of *E. coli* isolates (Fairbrother and Gyles, 2012).

Pathotype is the term used to classify *E. coli* by their virulence mechanisms. The broad classes identified include, Shiga toxin producing *E. coli* (STEC), enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC) and extraintestinal pathogenic *E. coli* (ExPEC) (Fairbrother and Gyles, 2012) (Table 1).

Enteropathogenic *E. coli* (EPEC)

Enteropathogenic *E. coli* is a pathotype found in post weaning diarrhoea of pigs. This bacterium possesses a

Table 1. Important pathotypes of pathogenic *Escherichia coli* in pigs and associated virulent traits.

Pathotype	Adhesins	Toxins
ETEC	F5(K99), F6 (987P), F41	STa
	F4(K88)	STa, STb, LT, EAST-1, α-hemolysin
	F4(K88), AIDA,	STa, STb, LT, EAST-1, α-hemolysin
	F18, AIDA	STa, STb, LT, Stx (VT), EAST-1, α-hemolysin
EPEC	Eae (intimin)	
STEC (VTEC)	F18, AIDA	Stx2e,(VT2e), EAST-1, α-hemolysin
	Eae (intimin)	Stx1 and/or Stx2
ExPEC	P,S	CNF
	P,S	CNF

complex secretion system that injects over 20 effector proteins into the host enterocyte. This allows intimate adherence of the bacteria into the pigs intestinal epithelium to develop a characteristic “attaching and effacing” (AE) lesion. The EPEC together with other *E. coli* pathotypes that result in AE are collectively known as attaching and effacing *E. coli* (AEEC) (Zhu et al., 1994).

Shiga toxin producing *E. coli* (STEC)

Shiga toxin producing *E. coli,* produce a family of cytotoxins known as Shiga toxin (Stx) or verotoxin (VT). Many STEC are not pathogenic in the intestinal flora but when they possess additional virulence traits, they become highly pathogenic (Fairbrother and Gyles, 2012).

In pigs, the most pathogenic STEC are those that cause oedema disease known as oedema disease *E coli*, with apostrophes before and after as above (EDEC). EDEC produces stx2e and F18ab or F18 ac (DebRoy et al., 2009). Another subgroup of STEC is the enterohaemorrhagic *E. coli* (EHEC) which also possess eae and the same secretion system as EPEC (Fairbrother and Gyles, 2012). However, production of Shiga toxins alone may not be sufficient for *E. coli* O157:H7 pathogenicity (Mead and Griffin, 1998). Other virulence factors such as the intimin protein (involved in the attachment of the *E. coli* O157 to enterocytes), the presence of a plasmid encoded hemolysin, or both, are important in the pathophysiology of haemorrhagic disease (Mead and Griffin, 1998).

Extra intestinal pathogenic *E. coli* (ExPEC)

Extra intestinal pathogenic *E. coli* are a group of heterogeneous *E. coli* in the intestinal tract of pigs that can invade other systems to cause bacteraemia resulting in septicaemia or localised infections such as meningitis and arthritis (Fairbrother and Ngeleka, 1994). The ExPEC possess lipopolysaccharides which protect the bacteria from being killed by serum complement and phagocytes (Fairbrother and Gyles, 2012).

Enterotoxigenic *E. coli* (ETEC)

The ETEC pathotype is the most important among the pathogenic *E. coli* producing one or more enterotoxins that induce secretory diarrhoea in pigs (Fairbrother and Gyles, 2006).This pathotype produces two major enterotoxins; heat stable toxin (ST) and heat labile toxin (LT) which are both further subdivided into STa, STb, LTI and LTII, respectively (Evans et al., 1972; Czirók et al., 1992).The ETEC that causes neonatal diarrhoea produces only STa and possess one or more fimbriae F4 (K88), F5 (K99), F6 (987P) and F41 (Fairbrother and Gyles, 2012). Similarly, ETEC that causes post-weaning diarrhoea produces STa, STb, LT, and enteroaggregative heat stable enterotoxin (EAST-1) (Zhang et al., 2007) while ETEC isolates that produces STb or STb: EAST-1 from weaned pigs may also produce an adhesion involved in diffuse adherence (AIDA-I) (Mainil et al., 2002; Ngeleka et al., 2003; Niewerth et al., 2001).

Enterotoxigenic *E. coli* causes an estimated 840 million gastrointestinal infections and about 380,000 deaths worldwide each year in pigs (Gupta et al., 2008), leading to substantial economic losses for swine producers worldwide (Nagy and Fekete, 2005). The bacteria adhere to and colonize the intestinal mucosa of the small intestine (jejunum, ileum and to a lesser extent, the duodenum) (Arbuckle, 1970; Cox and Houvenaghel, 1993). They also adhere to enterocytes using surface fimbriae (pili) that adhere to specific receptors on enterocytes, without inducing morphological lesions but elaborate enterotoxins that act locally on enterocytes, leading to fluid secretion resulting in the exacerbation of the diarrhoeal illness in pigs (Verbrugghe et al., 2015). A very important illness induced by ETEC toxins is post-weaning diarrhoea in piglets (Verbrugghe et al., 2015).

E. coli post-weaning diarrhoea (PWD)

Post-weaning diarrhoea, also known as post-weaning enteric colibacillosis, is an important cause of death in weaned pigs worldwide. Infection usually occurs during the first weekpost weaning and often results in decreased weight gain (Taylor, 2013). Several factors, such as the stress of weaning, lack of antibodies originating from the sow's milk and dietary changes, contribute to the severity of the disease, manifesting as sudden death or severe diarrhea (Fairbrother, 1999; Amezcua et al., 2002; Maynard et al., 2003).

Most outbreaks have occurred in early-weaned piglets although traditional herds are being increasingly affected (Fairbrother, 1999; Amezcua et al., 2002; Maynard et al., 2003).

Virulence factors of *E. coli* associated with post-weaning diarrhea

Post-weaning diarrhea is caused primarily by ETEC, a pathotype that is characterized by the production of adhesins and alpha-hemolysin, which produce colonies with clear zones of haemolysis on blood agar. Several studies have shown that *E. coli* isolated from weaned pigs with diarrhoea were haemolytic (Frydendahl et al., 2003; Chen et al., 2004).

Alpha-hemolysin, an approximately 110 kDa pore-forming cytolysin, belongs to the RTX family of toxins. The hlyA gene that encodes the hemolysin is part of an operon that is found on plasmids in ETEC. It is a potent cytotoxin that can damage a variety of cells (Frydendahl, 2002). Serological typing has been expanded to include fimbrial antigens, which are virulence factors, as well as O and H antigens which are virulence markers (Chen et al., 2004). Some strains of ETEC that cause PWD possess additional genes that encode Shiga toxin 2e (Stx2e), allowing them to cause edema disease (ED). The ETEC strains that produce Stx (VT) are appropriately called ETEC/STEC or ETEC/VTEC (Nagy and Fekete, 1999).

Enteropathogenic *E. coli* have also been implicated in PWD (Zhu et al., 1994; Zhu et al., 2010; Janke et al., 1989; An et al., 2000). Identification of porcine EPEC (PEPEC) is challenging and veterinary diagnostic laboratories do not routinely seek to identify this pathotype of *E. coli* (Fairbrother, 1999). The eae (*E. coli* AE) gene is a marker for PEPEC, but some eae-positive porcine E. coli isolates may be non-pathogenic. O45 serogroup has been shown to possess genes of the locus of enterocyte effacement (LEE); a locus well established to confer ability for AE lesions (Zhu et al., 1994; An et al., 2000; Helie et al., 1991). Immunity to one strain of pathogenic *E. coli* does not essentially protect from others, while successive strains can pass through herds (Bertschinger, 1999).

Pathogenesis of post-weaning diarrhea

Post-weaning diarrhea is an enteric disease in pigs localised in the small intestine, where digesta flows quickly. The EPEC that causes this condition attaches to the enterocytes lining of the villi or to the mucus covering the villi with the fimbriae or pili, which prevents the bacteria from being flushed to the large intestine. Thereafter, the enterotoxigenic *E. coli* which have colonised the small intestine incites hypersecretory diarrhoea through the release of distinct enterotoxins such as the LT and ST. (Francis, 2002; Zhang et al., 2007).

The LT induces secretion of chloride ions, sodium ions, bicarbonate ions and water into the lumen by binding irreversibly to the mucosal cells and activating the adenylcyclase cyclic AMP system (Thiagarajah and Verkman, 2003; de Haan and Hirst, 2004, Fairbrother et al., 2005) while the ST (STa and STb) inhibits the absorption of sodium and chloride ions from the lumen into the epithelial cell via the guanylcyclase–cyclic GMP system, both resulting in fluid retention. Intestinal colonisation and diarrhoea typically last for about 4 to 14 days, with the organism being spread between animals by the faeco-oral route and aerosols (Bertschinger, 1999).

Pigs displaying PWD harbour massive numbers of haemolytic *E. coli* in the jejunum, whilst there is minimal change in numbers of other bacteria (Smith and Jones, 1963). It is common for EHEC to appear in the faeces of pigs in increased numbers, in the first week after weaning in both healthy and diarrhoeic pigs, although the numbers are higher in diarrhoeic pigs (Kenworthy and Crabb, 1963; Hampson et al., 1985) (Table 2).

The act of weaning is an essential precipitating factor for PWD, regardless of the age at weaning. All of the factors involved with weaning create an environment suitable for the proliferation of *E. coli* in the small intestine. Slower gut transit time and gut stasis immediately after weaning allow bacteria the opportunity to attach and time to multiply (Pluske et al., 2002). An inability of piglets to adequately thermoregulate, combined with sub-standard weaning accommodation, may result in cold stress. This alters intestinal motility and is thought to be a major factor in the pathogenesis of PWD (Wathes et al., 1989). Other factors include social stresses from mixing, fighting and crowding which trigger cortisol release, most likely increasing transit time and depressing the immune response to enhance bacterial infection; moving to a new pen increases the chance of exposure to microbes residing in fresh or dry matter in the environment; the presence of other pathogens such as rotavirus in the environment thereby increasing the likelihood and severity of disease (Lecce *et al.,* 1983). Poor hygiene will also increase the pathogenic *E. coli* load delivered to the small intestine because of faeco-oral cycling (Madec et al., 1998).

Table 2. *Escherichia coli* and its virulence profiles in pigs.

Country	Source	Sample	Pathotype	Serotype	Age(days)	Virulence factor	Summary of result	References
South Africa	Farm	Faeces and intestinal tissues	ETEC	-	35	EAST 1	*E. coli* associated endotoxaemia.	(Fasina et al.,2015)
China	lab samples	Bacterial isolates	ETEC and VTEC	-	-	F18+	F18+ is the main colonization factor for VTEC and ETEC.	(Cheng et al., 2005)
Norway	Abbatior	intestinal content	STEC	O157: H7		stx2, eae, *fli*C-H7	Prevalence of *E. coli* O157:H7 in pigs is low in Norway	(Johnsen et al., 2001)
US	Herd	Isolates	ETEC	-	14	K88, K99, 987P, ST, LT	ETEC produces k88,K99, 987P, LT and ST.	(Moon et al., 1980)
US	Slaughter facility	Colon(faeces)	STEC	O157: H7	-	stx1, stx2, eae, hly	Pigs in the US can habour *E. coli* O157:H7.	(Feder et al., 2003)
Denmark	Lab samples	intestinal content	PEC	O8, O45, O138, O139, O141, O147, O149 and O157	22	F4, F18, STa, STb, LT,ESAT1, VT2e	VTEC and ETEC in PWD and ED belong to limited serogroups and are haemolytic.	(Frydendahl, 2002)
US	Farm	faeces	STEC	-	140	stx1,stx2	The incidence of STEC in swine varies.	(Fratamico et al., 2004)
Canada	Farm	rectal swab	ETEC	O149	35	Sta, STb, LT, Vtx, F18 F4	PWECD is an economically important disease in pigs.	(Amezcua et al., 2002)
US	Farm	Faecal swabs, faeces or intestinal content	ETEC	-	35	K88, K99, STa, STb, LT, F18, F41, stx2e, EAST1, AIDA-1, paa, eae	Broad arrays of virulence genes are associated with PWD in pigs.	(Zhang et al., 2007)
China	Field isolates	Faecal swabs	-	O8, O9, O11, O20, O32, O91, O93, O101, O107, O115, O116 and O131	49	F4, F5, F6, F18, F41 STI, STII, stx2e	Pigs with PWD have *E. coli* enterotoxins and shiga toxin 2 variant.	(Chen et al., 2004)
Mexico	Farm	faeces swabs	-	-	11& 28	LT, STa, STb, Stx1, Stx2 and EAST 1, F4, F5, F6, F17, F18 and F41	There are a there wide variety of virulence genes associated with diarrhoea in piglets.	(Toledo et al., 2012)
Switzerland	Farm	intestinal content	ETEC and VTEC	O139, O141 and O149	56	F107 SLT-IIv, LTI, STIa, STII	F107 are a major colonisation factor in O139: K12 and O141: K85ab *E. coli* serogroups.	(Imberechts et al., 1994)
Japan	Necropsy specimen	intestinal content and rectal swab	ETEC	O149, O157, O141 and O8	26	Sta, LT, K88, K99, 987P, F41, 4F	There are clear differences in strains of ND and PWD in terms of ETEC strain, enterotoxin type and adhesins.	(Nakazawa et al., 1987)
Belgium	Farm	Serum	ETEC	-	392	F4	F4+ ETEC is highly prevalent and widely spread in non-vaccinated pig breeding farms in Belgium.	(Van den Broecket al.,1999)
India		Faeces	STEC and EPEC	O9, O20, O24, O59, O60, O85, O100, O103, O112, O113, O116, O118, O119, O123, O137 and O152	40	stx1, stx2, eaeA, hlyA	STEC and EPEC are associated with diarrhoea in piglets and infants.	(Begum et al., 2014)
Cuba	Farm	Fecal Isolates	EPEC	O141 and O157	30	STb, STa, VT2e, LT, F18, F6	ETEC and VTEC isolates from diarrhoic pigs belong to restricted number of serogroups and serotypes.	(Blanco et al., 2006)
India	Farm	Liver, Lung, Intestine (necropsy)	STEC	-	-	stx2, eae	eae and stx genes are the prime causes of oedema disease in pigs.	(Barman et al., 2008)
Germany	Field strains	Isolates	STEC and ETEC	-	-	F18ac, F18ab	The F18 fimbrial subtypes are significantly associated with pathovars of *E. coli* strains.	(Barth et al., 2011)
Denmark	Institute	Faeces	ETEC	O149 and O138,	21	-	Diarrhoea occurrence is associated with faecal shedding of haemolytic *E. coli*.	(Carstensen et al., 2005)

Table 2. Contd.

Vietnam	Farm	Faeces, intestinal content	ETEC	O149 and O8	16	F4, F5, Sta,STb,LT	A large number of ETEC isolates belong to O8 serogroup, producing Sta, STb and LT but lacked fimbriae genes.	(Do et al., 2006)
Canada	Farm	Lab isolates	EPEC	O8, O116, O147, O138 and O45, O4 and O98	29	-	82% of *E. coli* isolates were enteropathogenic by the ligated intestine test in pigs.	(Gyles et al., 1971)
Uganda	Farm	Faeces	ETEC and VTEC	-	-	F18+, F4, LT, STa, STb and Stx2e	ETEC and VTEC infections are common in central uganda but clinical cases are masked by management practices.	(Okello et al., 2015)
China	Farm	Liver, spleen, kidney, heart, mesenteric lymph node		O107, O101, O9, O60 and O26.		-	O107, O101, O9, O26, and O60 are the dominant serotypes in southern china.	(Chen et al., 2013)
Korea	Farm	ileal contents	EAEC, ETEC	-	14	EAST 1, F4, F5, F6, F41, STa, STb, LT.	EAST1 is prevalent in *E. coli* and it is a virulence determinant in the pathogenesis of enteric colibacillosis of pre-weaned pigs.	(Choi et al., 2001b)
US	Research and diagnostic lab	Lab specimens	ETEC	O8, O9, O20, O101, O141, O149, O157, O138, and O139.		K88, LT, Stx2e; F18, STa, and STb	Loss of virulence genes is not uncommon in ETEC.	(Francis, 2002)
Spain	Farm	Isolates	ETEC	08, 09, 020, 0101, 0141 and 0149	15	F6 (987P), F5 (K99), F4 (K88) and F41	F6 fimbriae were found in higher rates than F5, F4, and F41 in younger and older piglets with ETEC strains.	(Garabal et al., 1997)
US	Farm	Isolates	STEC and ETEC	O138, O139, O141 and O147		f18, sta, stb, and stx2	Serogroup O147 may be a common serotype of oedema disease-causing *E. coli* in the United States.	(Helgerson et al., 2006)
England	Farm	Intestinal content	ETEC	O149	28	LT	Weaning at three weeks of age did not precipitate a profound change in the *E. coli* intestinal flora in the post-weaning period.	(Hinton et al., 1985)
Bulgeria	Farm	faeces and intestinal content	EHEC; VTEC	-	52	F18, F4	In the 2–3 post-weaning weeks, toxin producing *E. coli* that possesses the adhesion factor F4 is responsible for diarrhoea.	(Lyutskanov, 2011)
Zimbabwe	Farm	Faecal swabs	ETEC , VTEC	-	13	STa, STb, LT, Stx-2e F4, F18, F5 and F41, and F6	Vaccination or vaccine development based on F4, F5, F6, F18, and F41 antigens continues to be appropriate for ETEC infections.	(Madoroba et al., 2009)
Canada	Farm	Fecal swabs, faeces or intestinal content	ETEC and EPEC	O7, O157, O149, O26, O69, O139, O141, O103, O108, O109,O119, O20, O21 and O22	29	EAST1, AIDA-I, F4, F5, F6, F18, LT,STb,STa, EAE, Paa	AIDA-I/STb was dominant and EAST-I may not be an important marker for diarrhoea in pigs.	(Ngeleka et al., 2003)
Hungary	Farm	Intestine	-	O8, O138, 0139, 0141, 0147, O149, and 0157	49	K88, STa, STb, LT and VT	73% of K88 isolates had the capability to produce enterotoxins or VT which could contribute to post-weaning diarrhoea in pigs.	(Nagy et al., 1990)
Japan	Farm	Faece, cerebrum, liver, lung, heart blood, kidney, intestinal content, mesenteric lymph node	ETEC	O149: H-, O149: H10, O15:H9, O111:H- and OR: H6	19	STa, LT, K88,	Growth of ETEC was not active in healthy weaning pigs but infection with PRRS virus results in ETEC systemically with per acute death.	(Nakamine et al., 1998)
Germany	Farm			O138, O139, O139, O141, O141, O147 andO149	-	stx2e, fedA, orfA,orfB, AIDA	Porcine *E. coli* strains are a major reservoir for AIDA genes.	(Niewerth et al., 2001)
Canada	Animal health laboratory	Isolates	ETEC	O149		estA (STa), STb, LT, astA (EAST1), K88ac	Recent O149 ETEC associated with PWD in pigs in Ontario are different from the old O149 ETEC, and the new isolates has estAgene.	(Noamani et al., 2003)

Table 2. Contd.

Vietnam	Farm	small intestine, mesenteric lymph node, liver, lung, and spleen	-	O139, O141, O138, and O149	28	VT2e+, fedA(F18), AIDA, Sta, STb	0139:K82+/VT2e+/F18+/AIDA+ seropathotype was predominant and antibiotic resistances is widely distributed in *E. coli* causing ED in northern Vietnam.	(Oanh et al., 2010)
Poland	Farm	Faeces or rectal swabs	-	O139, O141 and O138	35	Stx2e, F18, STI, STII.	Molecular characterization of *E. coli* using the RAPD polymorphism analysis is a quick and convenient method to differentiate *E. coli* bacteria of the same and different serogroups.	(Osek, 2000)
China	Farm	rectal swab	ETEC	O80, O141, O139, O6, O9, O20, O101, O93, O138, O147, O157, O38 and O45.	26	K88, K99, F41, F18, 987P, STa, STb, LT, Stx2e	Novel serogroups O80, O6 and O38 of *E. coli* in pigs were identified in western China.	(Qi et al., 2012)
south Africa	Research council	rectal swab	ETEC, STEC, EAEC	-	73	STa, STb, LT, Stx2e, EAST-1, PAA, AIDA-I, EAE	PAA and AIDA-1 are important in South African pigs.	(Mohlatlole et al., 2013)
Korea	Farm	caecal and ileal contents	ETEC, STEC, DAEC	-	-	AIDA1, F18ab, Stx2e, Sta, STb, EAST1	AIDA gene is not restricted to DAEC strains.	(Ha et al., 2003)
Brazil	Farm	swabs		-	-	F4, F5, F6, F18, F41, STa, STb, LT and STx2e	*E. coli* strains isolated from pigs with diarrhoea possessed the genes for LT or/and ST enterotoxins.	(Vidotto et al., 2009)
Slovakia	Farm	Rectal swab and intestinal content	ETEC	O8, O54, O84, O101, O141, O141, O147, O149, O163, O2, O15, O84 andO157.	14	LT, Sta, STb, Stx1, Stx2, F4, F18, F6, F5, F41, F17, eae, EAST1	There is high prevalence of ETEC that possess LT and STb genes and the F4 colonization factor in piglets with diarrhoea in Slovakia.	(Vu-Khac et al., 2007)
China	Farm	Faeces	ETEC	-	-	EAST1, irp2, paa, STb, AIDA-I, LT-I, ler, hlyA, K88, eae, STa, sepA, F18, afaD, afaE, K99 and Stx2e	Relatively few isolates from the study express K88, K99, LT-I or STa, but EAST1, irp2, AIDA-I, paa and STb were frequent in *E. coli* strains in suckling pigs with diarrhoea in China.	(Liu et al., 2014)
Slovakia	Farm	Intestinal content	ETEC	-	21	F4, F5, F6, F18, F41, STa, STb, LT, STx2e, EAST1	There is a wide distribution of the astA gene among *E. coli* strains isolated from diarrhoeic piglets in Slovakia and a strong association of the astA gene with F4-positive strains.	(Vu-Khac et al., 2004)
Czech Republic	Farm	Rectal swab and intestinal content	ETEC and STEC	O149	-	LT+, STa+, K88, F18, EAST-1, paa.	There is a significantly higher prevalence of astA positive *E. coli* isolates among apparently healthy piglets in comparison with diarrheic piglets.	(Zajacova et al., 2012)
China	Farm	Rectal swab	ETEC and STEC	O141, O9, O32, O2, O116, O107, O147, O139, O91, O45 and O98.	49	AIDA-I, Sta, Stb, Lt, Stx2e, EAST1, F5, F6, F18, F41	AIDA-I represents an occasional virulence factor for PWD and ED in pigs and has the potential to transfer between porcine and human *E. coli*.	(Zhao et al., 2009)
Spain	Farm	Reactal swab	ETEC and VTEC	-	15	STa, LT, VT, CNF1, CNF2, α-hemolysin.	Majority of piglets in this study that produced verotoxin also produced STa enterotoxin but CNF1 was produced from only 1.5% of sick piglets.	(Garabal et al., 1995)
Korea	Pathology department	Ileal and cecal contents	ETEC, STEC	-	-	EAST1, STa, STb, LT, Stx2e, F4, F5, F6, F18, F41.	*E. coli* carries east1 gene in high prevalence in weaned pigs with diarrhoea and/or edema disease.	(Choi et al., 2001a)

Table 2. Contd.

Korea	Farm	Jejunal, ileal and caecal contents	ETEC, STEC	-	-	STa, STb, LT, Stx2e, F4, F5, F6, F18, F41	Genes for F18 and Stx2e are prevalent among *E coli* isolated from post-weaning pigs with diarrhoea or oedema disease.	(Kwon et al., 2002)
Denmark	national vet laboratory	Intestinal content and faeces	-	O149, O139, O138,O101 O8, O64, O147, O141 and O157	4,15,36 and 56	STa, STb, LT, VT, K88, 987P , F107, K99 and F41	Results correlation between genotypic and phenotypic methods was 97.7-100%. VT and F107 genes were more frequent in post-weaning than in neonatal *E.coli* strains.	(Ojeniyi et al., 1994)
Canada	vet medicine faculty	intestinal content	ETEC	O8,O9, O101, O9, O20,O64,O10, O157, O147,O149, O115, O138,O139,O141,O45,O26, O119,O15 and O108	31	F4, F5, F6, F41, STa, STb, LT and VT	The most important pathotypes among enterotoxigenic isolates in this study were F4:LT:STb, F5:STa, STb, F5:F41:STa, F4:STb, F6, STa, and LT.	(Harel et al., 1991)
Brazil	Farm	Faeces	ETEC, STEC	-	11	LT-I, STa, SLT-I, SLT-II, SLTIIv, F18ac.	eaeA gene and intimin production and/or Shiga-like toxins may be an important cause of diarrhoea among piglets.	(Martins et al., 2000)
Poland	Farm	Faeces or rectal swabs	-	-	35	LTI, STI, Stx2e, F4 F5 F6 F17 F18 and F41	Low prevalence of fimbria-positive *E. coli* strains isolated from pigs with PWD was found in this study.	(Osek, 1999)
Poland	Farm	Rectal swabs	ETEC	O1, O8, O9, O66, O138, O141, O147 and O149	35	EAST1-1, F4, LTI STI and STII	EAST1 gene is widely distributed among *E. coli* strains isolated from piglets with post-weaning diarrhoea.	(Osek, 2003)
Poland	Farm	Faecal swabs	ETEC	-	5& 42	Sta, LT, STb, K88, K99, 987P, F1	Enterotoxigenic (LT and STb) *E.coli* from suckling and weaned piglets with diarrhoea were 90.5% and 69.1% respectively and 18.5% of strains from healthy piglets were STa.	(Osek and Truszczyński, 1992)
Poland	Farm	Feaces	-	O157, O149, O66, O138, O139 and O141	-	F4, fedA, eltI, estI, estII, stx1, stx2e	*E. coli* from pigs with post-weaningdiarrhoea (13 out of 21 isolates) or from oedema disease (16 out of 19 strains) are able to produce F18 fimbriae.	(Osek et al., 1999)
Argentina	Farm	Rectal swabs spleen, kidney and liver	ETEC and VTEC	O8, O9, O64, O101, O138, O139, O149 and O162		STIa, STb, LTI, VT2e, VT1, VT2all	ETEC strains predominate in the group of animals with diarrhoea, STIa prevails in ETEC from pigs with diarrhoea, O64 prevails among ETEC and O138 prevails for ETEC/VTEC strains.	(Parma et al., 2000)
Sweden	Farm	faeces and intestinal content	ETEC	O149, O101, O9, O20, and O8,	25	LT, ST, K88, K99, 987P, F41	Frequency of O149 has been reduced, while that of O101 has increased to the same level as that of O149 in sweden.	(Soderlind et al., 1988)
Indonesia	Farm	Rectal swab		O20, O9 and O141	14	987P, ST, K88, K99.	Mixed infections with *E. coli* bearing different fimbrial antigens occur both within a group of piglets and in a single piglet.	(Hirst and Patten, 1991)
Slovakia	Farm	intestinal content and rectal swab	ETEC	-	15	F4, F5, F6 and F41	The frequency of occurrence of individual types of adherence antigens is related to geographical location.	(Vu-Khac et al., 2004)
Brazil	-	-	-	O139, O8, O9, O15, O20, O82, O101, O110 and O153	-	Stx2e, F18ab, STI, STII, LTI	Enterotoxin genes detected in high frequency are responsible for diarrhoea seen in pigs with oedema disease,	(da Silva et al., 2001)

Vaccination against pathogenic bacteria

Vaccination against pathogenic bacteria has become necessary as an alternative control measure due to the development of different serotypes of bacteria and bacterial resistance to a wide range of commonly used antibiotics (Fairbrother et al., 2005). Frequently used vaccines against bacterial diseases in swin

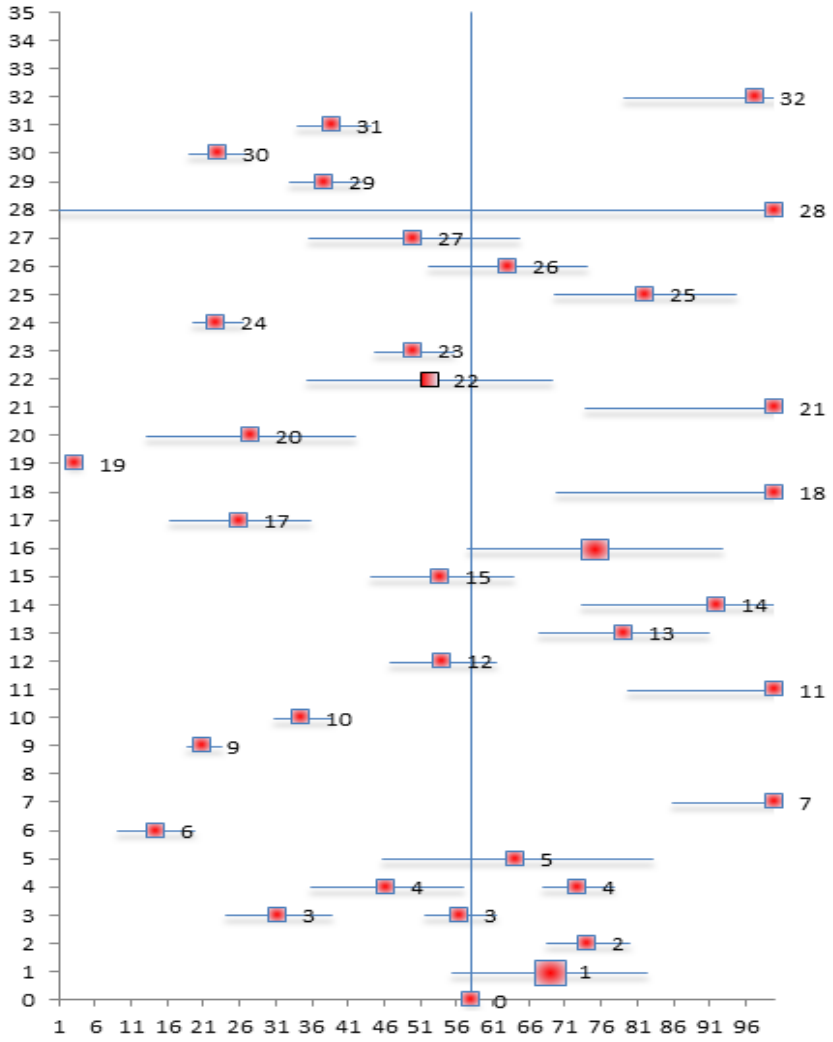

Figure 1. Forest plot of *prevalence of Escherichia coli* virulence factors

contain whole-cell killed micro-organisms, purified microbial components, or recombinant proteins (Haesebrouck et al., 2004). Vaccination against bacteria pathogens in pigs is directed towards either the extracellular bacteria or the exotoxin produced by the bacteria (Haesebrouck et al., 2004).

Exotoxins are produced within the bacterial cytoplasm. Some are excreted through the living cell wall, while others are released only by lysis of bacteria. In diseases caused by exotoxigenic bacteria, antibodies neutralizing that toxin play an important role in protection of the host against disease, provided they are able to prevent binding of the exotoxin to its receptor on the host cell. Vaccines containing the inactivated toxin (toxoid) or a non-toxic but antigenic recombinant protein derived from the exotoxin can be expected to provide protection against disease.

Antibodies generally mediate protection against the surface antigens and certain secreted antigens of extracellular bacteria. Cellular immunity may also play a role (Haesebrouck et al., 2004).

Oral immunization of piglets with live avirulent strains of bacteriais is a new vaccination strategy for bacterial diseases. An example is the administration of avirulent *E. coli* carrying the fimbrial adhesins or oral administration of purified F4 (K88) fimbriae.

Other approaches to control bacterial diseases include supplementation of the feed with egg yolk, antibodies from chickens immunized with F4 or F18 adhesins, breeding of F18 and F4 resistant animals, supplementation with zinc and/or spray-dried plasma, dietary acidification, phage therapy, or the use of probiotics. However, to date, no single strategy has proven to be totally effective (Fairbrother, 2005).

Results of meta-analysis

In the present review, 29 countries with documented records of cases of *E. coli* were included with the USA having the highest number of references followed by

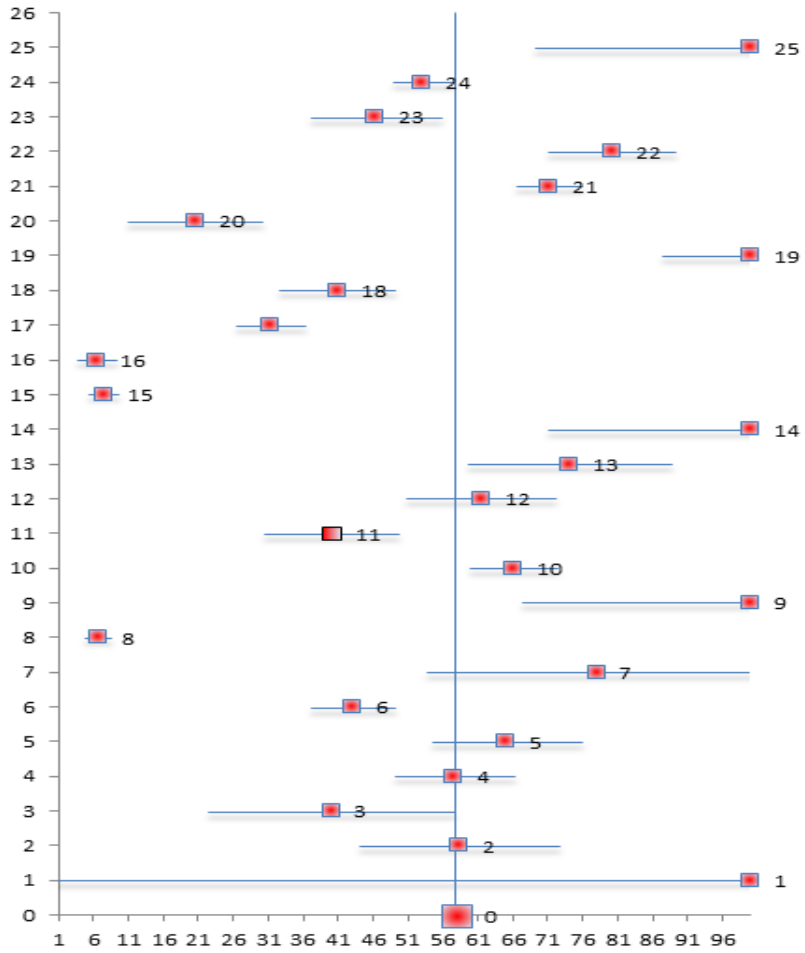

Figure 2. Forest plot for prevalence of *Escherichia coli* virulence factors in weaners.

China. About 74% of the samples were taken from farms and others were from samples submitted to research laboratories and veterinary faculties for necropsy.

In general 7 pathotypes were reported and 66.7% of the pathotypes identified were ETEC, 14.3% were ETEC and STEC, 7.9% were STEC, 31.7% were classified as ETEC/EPEC/EAEC, others were EHEC and DAEC (Table 2). Several serogroups were identified and the commonest were O141, O149, O139, O138, O8 and O9. 33.3% of samples collected were faecal swabs or faeces, 14.2% were intestinal segments, 17.4% were intestinal segments, feaces or rectal swabs and other organs, 6.3% were intestinal segments and other organs, 7.9% were lab isolates (Table 2). Piglets were 52.3%, 7.9% were porkers, 39.6% were weaners and all pigs were between 1 to 392 days old.

A total of 24,854 isolates were considered and 10477 were recorded as positives, the gene looked out for were STa, STb, LT, stx1, stx2, stx-2e F4, F5, F6, F18, F41, AIDA, EAST1, eae, paa, hlyA (Table 2). The diseases examined were diarrhoea in form of neonatal diarrhoea, colibacillosis, PWD and oedema disease. Some of the associated risk factors identified were poor housing, management and feed changes, extensive use of antibiotics as prophylaxis, overcrowding, high humidity and temperature changes (Table 2).

This study showed that India, USA, Japan, Slovakia and Denmark were the countries with the highest detection of virulence factors in piglets (100%; n = 3, 55, 42, 92 and 191 respectively), followed by Sweden (74%; n = 856) and Poland (72%; n =1125) (Table 3). For all the cases of virulence in piglets an overall prevalence of 57.93% (CI_{95}: 57.0 to 58.8) was estimated (n = 12970) (Table 3, Figure 1)

Similarly, South Africa, Cuba, Poland, Denmark, had the highest cases of virulence factor detection in *E. coli* in weaners (100%; n =2, 36, 46, and 240 respectively), followed by Canada (74.1% n = 135), the least detection was in found in China (6.5% n=324) (Table 4). The overall prevalence of virulence factor detection in weaners was 57.9% (CI_{95}: 56.99-58.83; n = 8058) (Table 4, Figure 2). Furthermore, in porkers the overall prevalence of *E. coli* virulence factor detection was36.45% (CI_{95}: 35.73- 37.57). The highest prevalence was found in the USA (70.5%; n = 687) and the lowest was in Norway (0.15% n= 1976) (Table 5, Figure 3).

Table 3. Prevalence of *Escherichia coli* virulence factors in piglets.

S/N	Study	Events	Sample Size	Outcome	SE	CI lower	CI upper	Forest Plot ID	Rate
1	US (herd)	108	111	0.972973	0.093624368	0.789469	1.156477	32	97.2973
2	Denmark (Diagnostic samples)	219	563	0.388988	0.026285344	0.337468	0.440507	31	38.89876
3	Mexico (Farm)	116	503	0.230616	0.021412186	0.188648	0.272584	30	23.06163
4	Japan (Necropsy samples)	214	567	0.377425	0.025800245	0.326857	0.427994	29	37.7425
5	India (Farm)	3	3	1	0.577350269	0.131607	2.131607	28	100
6	Denmark (Research institute)	45	90	0.5	0.074535599	0.35391	0.64609	27	50
7	Vietnam (Farm)	126	200	0.63	0.056124861	0.519995	0.740005	26	63
8	Canada (Farm)	164	200	0.82	0.064031242	0.694499	0.945501	25	82
9	Korea (Farm)	164	720	0.227778	0.017786456	0.192916	0.262639	24	22.77778
10	US (Research institute)	330	660	0.5	0.027524094	0.446053	0.553947	23	50
11	Spain (Farm)	36	69	0.521739	0.086956522	0.351304	0.692174	22	52.17391
12	US (Farm)	55	55	1	0.134839972	0.735714	1.264286	21	100
13	England (Farm)	14	51	0.27451	0.073365831	0.130713	0.418307	20	27.45098
14	Zimbabwe (Farm)	63	1984	0.031754	0.004000632	0.023913	0.039595	19	3.175403
15	Japan (Farm)	42	42	1	0.15430335	0.697565	1.302435	18	100
16	Germany (Farm)	27	104	0.259615	0.049963004	0.161688	0.357543	17	25.96154
17	Vietnam (Farm)	69	92	0.75	0.09028939	0.573033	0.926967	16	75
18	China (Farm)	112	208	0.538462	0.050879833	0.438737	0.638186	15	53.84615
19	Brazil (Farm)	92	100	0.92	0.09591663	0.732003	1.107997	14	92
20	Slovakia (Farm)	174	220	0.790909	0.059958663	0.67339	0.908428	13	79.09091
21	China (Farm)	206	381	0.540682	0.037671129	0.466847	0.614518	12	54.06824
22	Slovakia (Farm)	92	92	1	0.104257207	0.795656	1.204344	11	100
23	Czech Republic (Farm)	277	800	0.34625	0.020804146	0.305474	0.387026	10	34.625
24	Spain (Farm)	280	1334	0.209895	0.012543629	0.18531	0.234481	9	20.98951
25	Denmark (Research Laboratory)	191	191	1	0.072357461	0.858179	1.141821	7	100
26	Denmark (Research Laboratory)	28	194	0.14433	0.027275787	0.090869	0.19779	6	14.43299
27	Brazil (Farm)	45	70	0.642857	0.095831485	0.455027	0.830687	5	64.28571
28	Poland (Farm)	819	1125	0.728	0.025438379	0.678141	0.777859	4	72.8
29	Argentina (Farm)	70	223	0.313901	0.037518387	0.240365	0.387437	3	31.39013
30	Sweden (Farm)	634	856	0.740654	0.029415136	0.683001	0.798308	2	74.06542
31	Indonesia (Farm)	484	858	0.564103	0.025641026	0.513846	0.614359	3	56.41026
32	Slovakia (Farm)	74	160	0.4625	0.053764533	0.357122	0.567878	4	46.25
33	Brazil (Farm)	99	144	0.6875	0.06909635	0.552071	0.822929	1	68.75
-	-	-	-	0.579256	0.0047	0.570044	0.588468	Central Tendency	57.92558

Table 4. Prevalence of *Escherichia coli* virulence factors in weaners.

Serial number	Study	Events	Sample Size	Outcome	SE	CI lower	CI upper	Forest plot ID	Rate
1	south Africa (Farm)	2	2	1	0.707106781	-0.38593	2.385929	1	100
2	China (Lab samples)	63	108	0.583333	0.073493092	0.439287	0.72738	2	58.33333
3	Canada (Farm)	20	50	0.4	0.089442719	0.224692	0.575308	3	40
4	US (Farm)	175	304	0.575658	0.043515647	0.490367	0.660949	4	57.56579
5	China (Field isolates)	140	215	0.651163	0.0550333	0.543298	0.759028	5	65.11628
6	Mexico (Farm)	194	450	0.431111	0.030951974	0.370445	0.491777	6	43.11111
7	Switzerland (Farm)	39	50	0.78	0.12489996	0.535196	1.024804	7	78
8	India	48	720	0.066667	0.009622504	0.047807	0.085527	8	6.666667
9	Cuba (Farm)	36	36	1	0.166666667	0.673333	1.326667	9	100
10	Bulgeria (Farm)	409	619	0.660743	0.032671645	0.596707	0.72478	10	66.07431
11	Canada (Farm)	68	170	0.4	0.048507125	0.304926	0.495074	11	40

Table 4. Contd.

12	Hungary (Farm)	126	205	0.614634	0.054755962	0.507312	0.721956	12	61.46341
13	Canada (Animal health Lab)	100	135	0.740741	0.074074074	0.595556	0.885926	13	74.07407
14	Poland (Farm)	46	46	1	0.147441956	0.711014	1.288986	14	100
15	Korea (Farm)	45	604	0.074503	0.011106298	0.052735	0.096272	15	7.450331
16	China (Farm)	21	324	0.064815	0.014143752	0.037093	0.092537	16	6.481481
17	Korea (Pathology Department)	149	476	0.313025	0.025644024	0.262763	0.363287	17	31.30252
18	Korea (Farm)	94	230	0.408696	0.042153738	0.326074	0.491317	18	40.86957
19	Denmark (National Vet Lab)	240	240	1	0.064549722	0.873483	1.126517	19	100
20	Denmark (National Vet Lab)	17	83	0.204819	0.049675971	0.107454	0.302184	20	20.48193
21	Canada (Vet Medicine Faculty)	872	1226	0.711256	0.024086171	0.664047	0.758465	21	71.12561
22	Poland (Farm)	298	372	0.801075	0.046405044	0.710121	0.892029	22	80.10753
23	Poland (Farm)	96	207	0.463768	0.047333135	0.370995	0.556541	23	46.37681
24	Poland (Farm)	608	1146	0.530541	0.021516279	0.488369	0.572713	24	53.0541
25	Poland (Farm)	40	40	1	0.158113883	0.690097	1.309903	25	100
-	-	-	-	0.579062	0.0047	0.56985	0.588274	Central Tendency	57.90619

Table 5. Prevalence of *Escherichia coli* virulence factors in porkers.

S/No	Study	Events	Sample Size	Outcome	SE	CI lower	CI upper	Forest plot ID	Rate
1	Norway	3	1976	0.001518219	0.000876544	-0.0002	0.003236	1	0.151822
2	US	6	305	0.019672131	0.008031114	0.003931	0.035413	2	1.967213
3	US	484	687	0.704512373	0.03202329	0.641747	0.767278	3	70.45124
4	Belgium	95	135	0.703703704	0.072198477	0.562195	0.845213	4	70.37037
5	south Africa	106	263	0.403041825	0.039146883	0.326314	0.47977	5	40.30418
-	-	-	-	0.36648965	0.0047	0.357278	0.375702	Central Tendency	36.64897

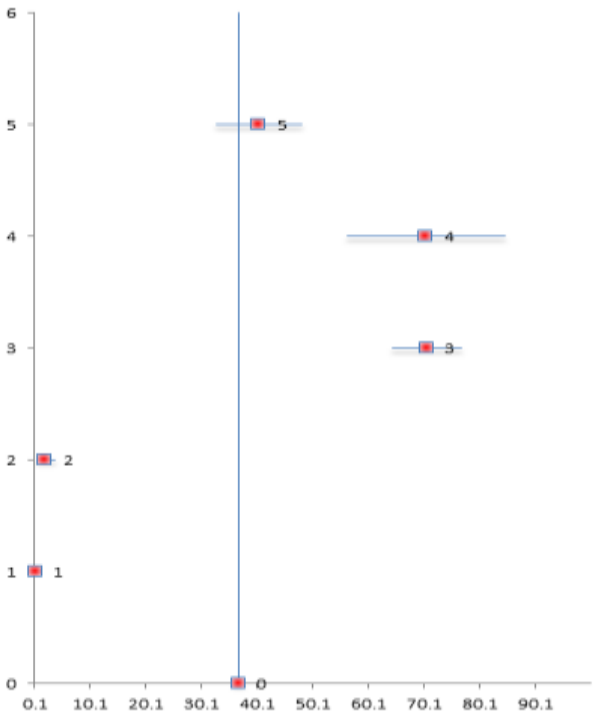

Figure 3. Forest plot for prevalence of *Escherichia coli* virulence factors in porkers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Supplementary Table 1. *Escherichia coli* virulence factors in piglets based on published information from different countries.

Location of study	Events	Sample Size	Outcome (es)	SE	Var	w	w*es	w*(es²)	w²	w_v	w_v*es	w_v*(es²)	w_v²
US (herd)	108	111	0.9730	0.0936	0.0088	114.083333	111	108	13015.0069	114.08333	111.00000	108.00000	13015.00694
Denmark (Diagnostic samples)	219	563	0.3890	0.0263	0.0007	1447.34703	563	219	2094813.43	1447.34703	563.00000	219.00000	2094813.431
Mexico (Farm)	116	503	0.2306	0.0214	0.0005	2181.11207	503	116	4757249.86	2181.11207	503.00000	116.00000	4757249.857
Japan (Necropsy samples)	214	567	0.3774	0.0258	0.0007	1502.28505	567	214	2256860.36	1502.28505	567.00000	214.00000	2256860.362
India (Farm)	3	3	1.0000	0.5774	0.3333	3	3	3	9	3.00000	3.00000	3.00000	9
Denmark (Research institute)	45	90	0.5000	0.0745	0.0056	180	90	45	32400	180.00000	90.00000	45.00000	32400
Vietnam (Farm)	126	200	0.6300	0.0561	0.0032	317.460317	200	126	100781.053	317.46032	200.00000	126.00000	100781.0532
Canada (Farm)	164	200	0.8200	0.0640	0.0041	243.902439	200	164	59488.3998	243.90244	200.00000	164.00000	59488.39976
Korea (Farm)	164	720	0.2278	0.0178	0.0003	3160.97561	720	164	9991766.81	3160.97561	720.00000	164.00000	9991766.805
US (Research institute)	330	660	0.5000	0.0275	0.0008	1320	660	330	1742400	1320.00000	660.00000	330.00000	1742400
Spain (Farm)	36	69	0.5217	0.0870	0.0076	132.25	69	36	17490.0625	132.25000	69.00000	36.00000	17490.0625
US (Farm)	55	55	1.0000	0.1348	0.0182	55	55	55	3025	55.00000	55.00000	55.00000	3025
England (Farm)	14	51	0.2745	0.0734	0.0054	185.785714	51	14	34516.3316	185.78571	51.00000	14.00000	34516.33163
Zimbabwe (Farm)	63	1984	0.0318	0.0040	0.0000	62480.254	1984	63	3903782136	62480.25397	1984.0000	63.00000	3903782136
Japan (Farm)	42	42	1.0000	0.1543	0.0238	42	42	42	1764	42.00000	42.00000	42.00000	1764
Germany (Farm)	27	104	0.2596	0.0500	0.0025	400.592593	104	27	160474.425	400.59259	104.00000	27.00000	160474.4252
Vietnam (Farm)	69	92	0.7500	0.0903	0.0082	122.666667	92	69	15047.1111	122.66667	92.00000	69.00000	15047.11111
China (Farm)	112	208	0.5385	0.0509	0.0026	386.285714	208	112	149216.653	386.28571	208.00000	112.00000	149216.6531
Brazil (Farm)	92	100	0.9200	0.0959	0.0092	108.695652	100	92	11814.7448	108.69565	100.00000	92.00000	11814.7448
Slovakia (Farm)	174	220	0.7909	0.0600	0.0036	278.16092	220	174	77373.4972	278.16092	220.00000	174.00000	77373.49716
China (Farm)	206	381	0.5407	0.0377	0.0014	704.665049	381	206	496552.831	704.66505	381.00000	206.00000	496552.8306
Slovakia (Farm)	92	92	1.0000	0.1043	0.0109	92	92	92	8464	92.00000	92.00000	92.00000	8464
Czech Republic (Farm)	277	800	0.3463	0.0208	0.0004	2310.46931	800	277	5338268.45	2310.46931	800.00000	277.00000	5338268.451
Spain (Farm)	280	1334	0.2099	0.0125	0.0002	6355.55714	1334	280	40393106.6	6355.55714	1334.0000	280.00000	40393106.6
Denmark (Research Laboratory)	191	191	1.0000	0.0724	0.0052	191	191	191	36481	191.00000	191.00000	191.00000	36481
Denmark (Research Laboratory)	28	194	0.1443	0.0273	0.0007	1344.14286	194	28	1806720.02	1344.14286	194.00000	28.00000	1806720.02
Brazil (Farm)	45	70	0.6429	0.0958	0.0092	108.888889	70	45	11856.7901	108.88889	70.00000	45.00000	11856.79012
Poland (Farm)	819	1125	0.7280	0.0254	0.0006	1545.32967	1125	819	2388043.79	1545.32967	1125.0000	819.00000	2388043.79
Argentina (Farm)	70	223	0.3139	0.0375	0.0014	710.414286	223	70	504688.457	710.41429	223.00000	70.00000	504688.4573
Sweden (Farm)	634	856	0.7407	0.0294	0.0009	1155.73502	856	634	1335723.43	1155.73502	856.00000	634.00000	1335723.427
Indonesia (Farm)	484	858	0.5641	0.0256	0.0007	1521	858	484	2313441	1521.00000	858.00000	484.00000	2313441
Slovakia (Farm)	74	160	0.4625	0.0538	0.0029	345.945946	160	74	119678.598	345.94595	160.00000	74.00000	119678.5975
Brazil (Farm)	99	144	0.6875	0.0691	0.0048	209.454545	144	99	43871.2066	209.45455	144.00000	99.00000	43871.20661
-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	--	-	-	-	-	-
K	16	-	-	-	Sums:	91256.4598	12970	5472	3980098538	91256.45979	12970	5472	3980098538
Df	15	-	-	-	-	-	-	-	-	-	-	-	-

Supplementary Table 1. Contd.

-		-	-	-	-	-	-	-	-	v	0.0758493	-	-
Q	3628.61	-	-	Q_v	3628.6138	-	-	-	-	-	-	-	-
I^2	99.5866	-	-	I^2_v	99.586619	-	-	-	-	-	-	-	-
-		-	-	-	-	-	-	-	-	-	-	-	-
es (fixed)	0.14213	-	-	es (random)	0.1421269	-	-	-	-	-	-	-	-
SEes (fixed)	0.00331	-	-	SEes (random)	0.0033103	-	-	-	-	-	-	-	-
CI (fixed)	0.13564	0.1486151	-	CI (random)	0.1356387	0.14861513	-	-	-	-	-	-	-

Supplementary Table 2. *Escherichia coli* virulence factors in weaners based on published information from different countries.

Location of study	Events	Sample Size	Outcome (es)	SE	Var	w	w*es	w*(es^2)	w^2	w_v	w_v*es	w_v*(es^2)	w_v^2
south Africa (Farm)	2	2	1.0000	0.7071	0.5000	2	2	2	4	2.00000	2.00000	2.00000	4
China (Lab samples)	63	108	0.5833	0.0735	0.0054	185.14286	108	63	34277.878	185.14286	108.00000	63.00000	34277.878
Canada (Farm)	20	50	0.4000	0.0894	0.0080	125	50	20	15625	125.00000	50.00000	20.00000	15625
US (Farm)	175	304	0.5757	0.0435	0.0019	528.09143	304	175	278880.56	528.09143	304.00000	175.00000	278880.56
China (Field isolates)	140	215	0.6512	0.0550	0.0030	330.17857	215	140	109017.89	330.17857	215.00000	140.00000	109017.89
Mexico (Farm)	194	450	0.4311	0.0310	0.0010	1043.8144	450	194	1089548.6	1043.81443	450.00000	194.00000	1089548.6
Switzerland (Farm)	39	50	0.7800	0.1249	0.0156	64.102564	50	39	4109.1387	64.10256	50.00000	39.00000	4109.1387
India	48	720	0.0667	0.0096	0.0001	10800	720	48	116640000	10800.00000	720.00000	48.00000	116640000
Cuba (Farm)	36	36	1.0000	0.1667	0.0278	36	36	36	1296	36.00000	36.00000	36.00000	1296
Bulgeria (Farm)	409	619	0.6607	0.0327	0.0011	936.82396	619	409	877639.13	936.82396	619.00000	409.00000	877639.13
Canada (Farm)	68	170	0.4000	0.0485	0.0024	425	170	68	180625	425.00000	170.00000	68.00000	180625
Hungary (Farm)	126	205	0.6146	0.0548	0.0030	333.53175	205	126	111243.43	333.53175	205.00000	126.00000	111243.43
Canada (Animal health Lab)	100	135	0.7407	0.0741	0.0055	182.25	135	100	33215.063	182.25000	135.00000	100.00000	33215.063
Poland (Farm)	46	46	1.0000	0.1474	0.0217	46	46	46	2116	46.00000	46.00000	46.00000	2116
Korea (Farm)	45	604	0.0745	0.0111	0.0001	8107.0222	604	45	65723809	8107.02222	604.00000	45.00000	65723809
China (Farm)	21	324	0.0648	0.0141	0.0002	4998.8571	324	21	24988573	4998.85714	324.00000	21.00000	24988573
Korea (Pathology Department)	149	476	0.3130	0.0256	0.0007	1520.6443	476	149	2312359.1	1520.64430	476.00000	149.00000	2312359.1
Korea (Farm)	94	230	0.4087	0.0422	0.0018	562.76596	230	94	316705.52	562.76596	230.00000	94.00000	316705.52
Denmark (National Vet Lab)	240	240	1.0000	0.0645	0.0042	240	240	240	57600	240.00000	240.00000	240.00000	57600
Denmark (National Vet Lab)	17	83	0.2048	0.0497	0.0025	405.23529	83	17	164215.64	405.23529	83.00000	17.00000	164215.64
Canada (Vet Medicine Faculty)	872	1226	0.7113	0.0241	0.0006	1723.711	1226	872	2971179.6	1723.71101	1226.00000	872.00000	2971179.6
Poland (Farm)	298	372	0.8011	0.0464	0.0022	464.37584	372	298	215644.92	464.37584	372.00000	298.00000	215644.92
Poland (Farm)	96	207	0.4638	0.0473	0.0022	446.34375	207	96	199222.74	446.34375	207.00000	96.00000	199222.74
Poland (Farm)	608	1146	0.5305	0.0215	0.0005	2160.0592	1146	608	4665855.8	2160.05921	1146.00000	608.00000	4665855.8

Supplementary Table 2. Contd.

Poland (Farm)	40	40	1.0000	0.1581	0.0250	40	40	40	1600	40.00000	40.00000	40.00000	1600
K	16	-	-	-	Sums:	35706.95	8058	3946	220994363	35706.95028	8058	3946	220994363
Df	15	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	v	0.0715685	-	-
Q	2127.5483	-	-	Q_v	2127.548312	-	-	-	-	-	-	-	-
I^2	99.294963	-	-	I^2_v	99.29496313	-	-	-	-	-	-	-	-
es (fixed)	0.2256704	-	-	es (random)	0.225670351	-	-	-	-	-	-	-	-
SEes (fixed)	0.005292	-	-	SEes (random)	0.005292046	-	-	-	-	-	-	-	-
CI (fixed)	0.2152979	0.23604	-	CI (random)	0.215297941	0.2360428	-	-	-	-	-	-	-

Supplementary Table 3. *Escherichia coli* virulence factors in porkers based on published information from different countries.

Location of study	Events	Sample Size	Outcome (es)	SE	Var	w	w*es	w*(es^2)	w^2	w_v	w_v*es	w_v*(es^2)	w_v^2
Norway	3	1976	0.0015	0.0009	0.0000	1301525.3	1976	3	1.694E+12	51.06604	0.07753	0.00012	2607.7405
US	6	305	0.0197	0.0080	0.0001	15504.167	305	6	240379184	50.90039	1.00132	0.01970	2590.8494
US	484	687	0.7045	0.0320	0.0010	975.14256	687	484	950903.02	48.52671	34.18767	24.08563	2354.8415
Belgium	95	135	0.7037	0.0722	0.0052	191.84211	135	95	36803.393	40.33179	28.38163	19.97226	1626.6533
south Africa	106	263	0.4030	0.0391	0.0015	652.53774	263	106	425805.5	47.36150	19.08867	7.69353	2243.1118
-	-	3366	0.3664897	-	-	-	-	-	-	-	-	-	-
K	16	-	-	-	Sums:	1318849	3366	694	1.694E+12	238.18643	82.736812	51.771239	11423.197
Df	15	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	v	0.0195817	-	-
Q	685.40921	-	-	Q_v	23.031651	-	-	-	-	-	-	-	-
I^2	97.811526	-	-	I^2_v	34.872232	-	-	-	-	-	-	-	-
es (fixed)	0.0025522	-	-	es (random)	0.3473616	-	-	-	-	-	-	-	-
SEes (fixed)	0.0008708	-	-	SEes (random)	0.064795	-	-	-	-	-	-	-	-
CI (fixed)	0.0008455	0.0042589	-	CI (random)	0.2203634	0.4743598	-	-	-	-	-	-	-

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