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# Prevalence, Antigenic Recognition and IgG Antibody Response in Pigs Infected with *Sarcoptes Scabiei* var. *Suis* in Confined Pens

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## ABSTRACT

Hog mange is a serious pruritic dermatitis caused by the itch mite *Sarcoptes scabiei* var. *suis*. The itch mite has remained a severe ectoparasite of utmost importance to the pig industry, especially with the environment promoting its spread. The ubiquity cum difficulty in diagnosing and treatment has led to great economic losses. This study aimed at evaluating the prevalence and antigenic recognition spectra of confined pigs in a resource poor community in Ogbomosho, South-West Nigeria.

Skin scrapings were obtained from the inner ears and skin of mange-established pigs to check for presence of mites. Enzyme-linked immunosorbent assays (ELISA) were carried out on serous blood obtained from naïve (uninfected) and mange-positive pigs. Autoantibody titres eliciting immunoglobulin G (IgG) binding to certain iron-binding proteins, namely,transferrin, ferritin, albumin, lactoferrin, haemoglobin, and haptoglobin, were measured at specific optical densities ($OD_{450}$). One hundred and fifteen (115) pigs comprising males (40, 34.8%) and females (75, 65.2%) were enrolled for the study. The prevalence of infestation was high (63, 54.7%) with manifestation of mange occurring in all growth stages: piglets (11, 42.3%), growers (23, 63.9%), and adults (29, 54.7%). Mange-positive pigs showed higher mean $OD_{450}$ to transferrin (0.49), ferritin (0.37), albumin (0.28), and lactoferrin (0.40). Both naïve and mange-positive pigs produced $OD_{450}$ to haemoglobin (0.07 and 0.15) and haptoglobin (0.26 and 0.27) indicating that IgG does not cause sequestration with those proteins even after infection. The presence of autoantibodies specific to sarcoptic mange will certainly open an array of clinical diagnostics and provide an avenue for new antiscabies drugs.

**Keywords:** Mange, Antigenic recognition spectra, Transferrin, Ferritin, Albumin, Lactoferrin, Hemoglobin, Haptoglobin, IgG

## INTRODUCTION

The etiologic agent of scabies/mange (*Sarcoptes scabiei*) is a common, widespread, highly contagious, and mite-caused skin disease of mammals (Bornstein et al., 2001). Separate *Sarcoptes* species have been described from a wide variety of domestic animals including man;they are morphologically indistinguishable and may represent physiological sibling species (Burgess, 1991; Dagleish et al., 2007; Shelley & Bart, 2007; Chhabra & Pathak, 2011). An immature gravid female mite cuts rapidly with the mouthparts and completely embeds itself within the horny layer of the skin of its host creating tunnels and laying eggs along its burrows (Rampton et al., 2013; Laha et al., 2014). Mass infestation causes significant morbidity and mortality in wild, domestic, and farm animals (Dagleish et al., 2007; Walton & Currie, 2007; Eo et al., 2008; Chhabra & Pathak, 2011). Secretory and excretory products deposited by the mites result in pruritus and other secondary infections,which include erythematous eruptions, papule formation, seborrhoea, and alopecia (Bornstein et al., 2001; Das et al., 2010). In time past, Sarcoptic mange in pigs was previously reported as a controllable infestation, but today, it has made significant impacts on intensive pig production (Arends et al., 1990; Davis &Moon, 1990; Cargill & Davies, 1999; Damriyasa et al., 2004; Chhabra & Pathak, 2011; Averbeck et al., 2014). *S. scabiei* var. *suis* infestation in swine causes considerable discomfort, a depressed growth rate, and reduced feed conversion efficiency (Davies, 1995; Firkins et al., 2001). The manifestation of the signs and symptoms of swine mange depends upon a complex interplay between a mite population that produces antigens and the immunological response of the host (Davis & Moon, 1990; Bornstein & Zakrisson, 1993; Rampton et al., 2013; Toet et al., 2014). The host immunological response to mite products and the ensuing pathological alterations are triggered off by the following: lymphokines, complement subunits, histamine, and serotonin released from mast cells and basophils (Reunala et al., 1984). Very few reliable diagnostic methods are in practice for identifying sarcoptic mange in swine (Rampton et al., 2013). The physical and laborious identification of mites, eggs, or feces from scrapings of infested skin or by identification of mite burrows still remains the gold standard. In the literature, a 100% specificity and <50% sensitivity have been observed in experimental field situations (Löwenstein et al., 2004; Walter et al., 2011; Rampton et al., 2013; Laha et al., 2014). However, a multitude of uncharacteristic manifestations sharing similar dermatological conditions with scabies/mange makes appropriate clinical diagnosis difficult;thus, specificity may indicate problems associated with under- or overdiagnosis (Shelley & Bart, 2007; Rampton et al., 2013; Arlian et al., 2015). To overcome the aforementioned shortcomings in clinical diagnosis,it becomes mandatory to include serodiagnostic investigation, which most often demonstrates affinity for specific antibodies to *S. scabiei* var. *suis* (Bornstein & Zakrisson, 1993; Bornstein & Wallgren, 1997; Hollanders et al., 1997; Kessler et al., 2003; Toet et al., 2014; Arlian et al., 2015). Plasma B cells have been isolated as the precursor for the release of Immunoglobulin G (IgG) molecules since it is the most common type of antibody in serum (≈75%) (Kuhn et al., 2008). Thus, the measurement of IgG in scabies-infected animals is a veritable diagnostic tool, and in reality, host IgG has been isolated in the anterior midgut of fresh mites (Rapp et al., 2006; Willis et al., 2006). Despite the inability of the mite to feed on occult blood, immunoglobulins do gain access to its midgut via diffusion from host capillary beds or from plasma cells (Arlian et al., 1994). In all *S. scabiei* varieties (*suis, canis*, and *hominis*), there have been some form of antigenic

relationships and responses against a range of specific epitopes (Arlian et al., 1996; Zalunardo et al., 2006; Rampton et al., 2013).The confirmation of IgG autoantibodies against transferrin in naïve and mange-positive pigs (Toet et al., 2014)and the use of enzyme-linked immunosorbent assays (ELISAs) and dot blot assays have further opened a new array of diagnostic potentials that needs to be explored in our settings to justify the generality of cross-immunoreactivity to natural autoantibodies. This study aims to investigate the prevalence of *Sarcoptes scabiei* var. *suis*, determine antigenic recognition spectra and identify IgG-binding proteins in sera of pigs housed in confined pens.

## MATERIALS AND METHODS

### Study Area

The study was conducted in five pig farms strategically located in the five local government areas (LGA) of Ogbomoso, South-West Nigeria, namely, Ogbomoso North and South, Surulere, Orire, and Ogo-oluwa LGAs. Ogbomoso is the second largest town in Oyo State with an urban population of about 334,000 (National Population Commission, 2007) and lies on coordinates of latitude 80° 07′ north of the equator and 40° 30′ east of the Greenwich Meridian at an elevation of 347 m (1,138 ft). It has an area landmass covering about 37,984 $km^2$ and lies within the derived tropical savannah region. Ogbomoso is the gateway to northern Nigeria;it is 57 km southwest of Ilorin and 53km northeast of Osogbo. Inhabitants are mostly Yoruba, with few migrants from the Hausa and Ibo ethnic groups. It is an agrarian community with plantations of yam, cassava, maize, tobacco, and other cash crops (Chernow et al., 1993). The farms selected for this study were commercial smallholder pens. At the time of sampling for this study, 25 commercial farms were identified in the study area. Only the farms that meet our study protocols was included, that is, piggeries that had enclosures with both bare and cemented floors.

### Ethical Approval

The protocols for this study were submitted for ethical consideration, and approval was given by the University of Ilorin Ethical Review and Consideration Committee. The respective owners of the sampled piggeries were contacted and enlightened concerning the protocols. The farmers' consent was requested, and an approval was given before commencement of the study.

### Study Design

A simple random sampling technique was employed in the selection of one piggery from each of the five LGAs that make up Ogbomoso environs. Only piggeries that were managed in enclosed fences were recruited for the study. Prior to sample collections, information regarding breed, age, sex, and last administration of topical antiscabies formulations was obtained from the farmers. The pig species were further ascertained following the morphological identification parameters as stated in Food and Agriculture Organization (2011) and Shannon et al. (2001). Only pigs confined in pens were sampled for this study. Age groupings of the pigs were done using the following categories: ≤6 months: piglets; 7–12 months: growers; ≥13 months: adults.

### Collection of Skin Scrapings and Identification of Mites

Skin scrapings were collected from the pigs that showed outward clinical signs of infestation (pruritus, red papules on skin, hair loss, and itching; Laha et al., 2014). The dry scraping technique as described by Fthenakis et al. (2000) was employed. Briefly, skin scrapings from three lesion sites

bordering healthy tissue were obtained from each animal, and approximately 1 $cm^2$ at each site was scraped. A scalpel blade was dipped into glycerin; a skin fold was pinched between the forefinger and the thumb and, whilst the blade was held at right angle to the skin, scraped until blood seeped from the abrasion. Skin crusts were collected into a sterile tube and subsequently dissolved in potassium hydroxide (KOH) following the guidelines set by Alonso et al. (1998). Samples for microscopic examination were incubated with 1M KOH at 65°C for 45–60 min. Following incubation, the solutions were centrifuged at 500rpm. Two drops of the solution were placed on a microscope slide, covered with a cover slip, and examined under 10×magnification on a stereo microscope (Olympus stereo microscope, SZ61). Hyperkeratotic mange was observed following the methods of Rambozzi et al. (2007) and Pence and Ueckermann (2002).

**Collection of Occult Blood**

Only pigs with ominous signs of hyperkeratotic mange were picked for the evaluation as positives (one from each farm, $n$ = 5) and naïve pigs (one from each farm, $n$ = 5). Approximately 2ml of blood was collected according to guidelines set by Framstad et al. (1988) from the external jugular vein of both the mange-positive and naïve (negative) pigs. The pigs were kept upright and held using a snout rope behind their canines, and their necks were stretched upwards. Occult blood was drained into EDTA-sampled bottles from the jugular veins.

**Western Blot Analysis**

Iron-binding lyophilized proteins were obtained from NIMER, Lagos (ProSci Incorporated, Poway). The content consists of transferrin, albumin, and ferritin. These serum proteins were appropriately dissolved in deionized water. About 5µg of the trio was separately added to 10µl, 0.5M Tris-HCl, pH 7.0, 120nM dithioethreitol (DTT) (sigmaaldrich.com), 0.5% w/v bromophenol blue, 0.5% w/v SDS, and 20% w/v glycerol. The prepared samples were electrophoresed according to guidelines by Bollag et al. (2002). Gels were subsequently equilibrated in a combination of 0.3% w/v Tris, 1.5% w/v glycine, 20% w/v methanol in deionized water for 15 min. Afterwards, the entire contents were transferred to an Immobilin-P transfer membrane called Polyvinylidine difluoride (Merck Millipore, Saint-Quentin-en-Yvelines, CEDEX France). Electro-transfer as described by Towbin et al. (1979) was then carried out. The membranes were washed with PBS-T and subsequently blocked with 5% w/v skimmed milk overnight at 27°C. The membranes were subjected to cleaning at least three times with PBS-T. One milligram per milliliter of purified pig antibody was diluted in 1/100 PBS-T and incubated for 1hr. A repeated wash of the membrane was carried out for 5 min with PBS-T and incubated in rabbit anti-pig IgG-HRP (http://www.thermoscientific.com). The contents were then diluted in 1/5000 PBS-T for 1hr at room temperature. After incubation, the membranes were subjected to repeat rinsing thrice in PBS-T for 15 min in each well and then washed once in deionized water. A combination of metal enhancers and 3, 3'- diaminobenzidine (DAB) (Sigma-Aldrich) wasused in developing the membranes.

**ELISA Test**

Indirect ELISAs were carried out on the sera collected from both pig positive and negative for mange after the methods of (Toet et al., 2014). Briefly, serum proteins obtained from the National Institute of Medical Research (NIMER, Animal lab), Yaba, Lagos, was diluted to a concentration of 10µg/ml with carbonate buffer (0.015M $Na_2CO_3$ and 0.035M $NaHCO_3$ at pH 9.6). The following iron-binding proteins (antigens) were tested:

transferrin (bovine, human/dog), ferritin (horse spleen), hemoglobin (bovine), albumin (bovine serum albumin [BSA]/alternatively skimmed milk produced by West Africa Milk Company, Nigeria [WAMCO]), lactoferrin (bovine), and haptoglobin (human). Each well of a 96-well ELISA microplate was coated with 50µl of antigen solution and subsequently incubated all through the night at 4°C for 10hr. The plates were washed three times with phosphate buffer saline (PBS) containing Tween 20 (PBS-T; 16-mM and 5-nM $NaHPO_4$, 120-mM NaCl, 0.05% (v/v) Tween 20 at pH 7.4) between each step. Plates were blocked for 2hr at room temperature with 200µl of PBS-T containing 3% (w/v) BSA. Sera were diluted with 1:2000 in PBS-T containing appropriate blocking agent and incubated at 27°C. Each serum was tested in triplicate on each plate and subsequently incubated at 37°C for 1hr. Secondary antibodies (SA; rabbit anti-pig IgG-horse radish peroxidase (HRP) antibody, Bethyl Laboratories, Inc., Montgomery, TX 77356, USA) were diluted 1:2000 in PBS-T containing appropriate blocking agents and incubated at 27°C for 30 min then mixed together in a rotary machine for proper content mixing. To each well, 50µl of SA HRP-PBS-T was added and incubated for 1hr at 37°C. The plates were washed three times for 15 min initially with PBS-T and subsequently with deionized water running through an improvised tap for 15 min. To each well, a 50-µl volume of the substrate 3,3',5,5'–Tetramethylbenzidine (TMB; Invitrogen) was then added, and wells were incubated in an opaque medium at 27°C for 15 min, and later, 50µl of 1-M HCl was added per well. After 5min, the plates were read with a microplate reader at an absorbance rate of 450nm.

### Analysis of Data

Data was entered and analyzed using SPSS (Statistical Package for Social Sciences) software statistical package version 16.0 to evaluate the mean $OD_{450}$ of the iron-binding proteins. Their mean differences were analyzed by comparisons made with the use of a one-way ANOVA. The level of statistical significance set at $p < 0.05$. From the values obtained, the cutoff values between negative and positive results were determined by the average result of the mange-negative animals added to three times the standard deviation obtained from the means. Sensitivity and specificity for each assay were determined following standard methods.

## RESULTS AND DISCUSSION

A total of 115 pigs comprising 40 males (34.8%) and 75 females (65.2%) were surveyed in the study area. The pigs were stratified into growth stage categories (i.e., piglets:≤6 months,growers: 7–12 months, and adults: ≥13 months), and these include 26 piglets (22.6%), 36 growers (31.3%),and 53 adults (46.1%).Out of the total population sampled for mange, 63 (54.8%) were infected and were thus distributed:11(42.3%) piglets, 23 (63.9%) growers, and 29 (54.7%) adults (Table 1).

The mean optical densities ($OD_{450}$) of the iron-binding proteins at 450nm and their respective standard deviations are depicted in Figure 1. Recorded IgG responses in mange-positive pigs were highest in transferrin (a maximum $OD_{450}$value of 0.64 and a minimum $OD_{450}$ value of 0.43) and lowest in hemoglobin (maximum $OD_{450}$ value of 0.18 and minimum $OD_{450}$ value of 0.10). Naïve pigs showed similar responses to all the iron-binding proteins. There were considerable differences between responses to transferrin, lactoferrin, albumin, and ferritin in the mange-positivepigs as compared to the naïve pigs. However, both mange-positive and naïve pigs produced similar responses in their IgG reactivity to hemoglobin and haptoglobin.

Table 1.Prevalence of *Sarcoptes scabiei* var. *suis* in Pigs in Confined Pens in Ogbomosho, South-Western Nigeria

| Growth Stage | No Examined | No Infected (%) | *p*-Value |
|---|---|---|---|
| Piglets | *26* | *11 (42.3)* | <0.001 |
| Male | *10* | *10 (38.5)* | |
| Female | *16* | *1 (3.8)* | |
| Growers | *36* | *23 (63.9)* | 0.644* |
| Male | *7* | *5 (71.4)* | |
| Female | *29* | *18 (62.1)* | |
| Adults | *53* | *29 (54.7)* | 0.378* |
| Male | *23* | *11 (47.8)* | |
| Female | *30* | *18 (60.0)* | |
| Total | *115* | *63 (54.8)* | |
| *p*-Value | *0.242** | | |

*Not significant at 0.05.

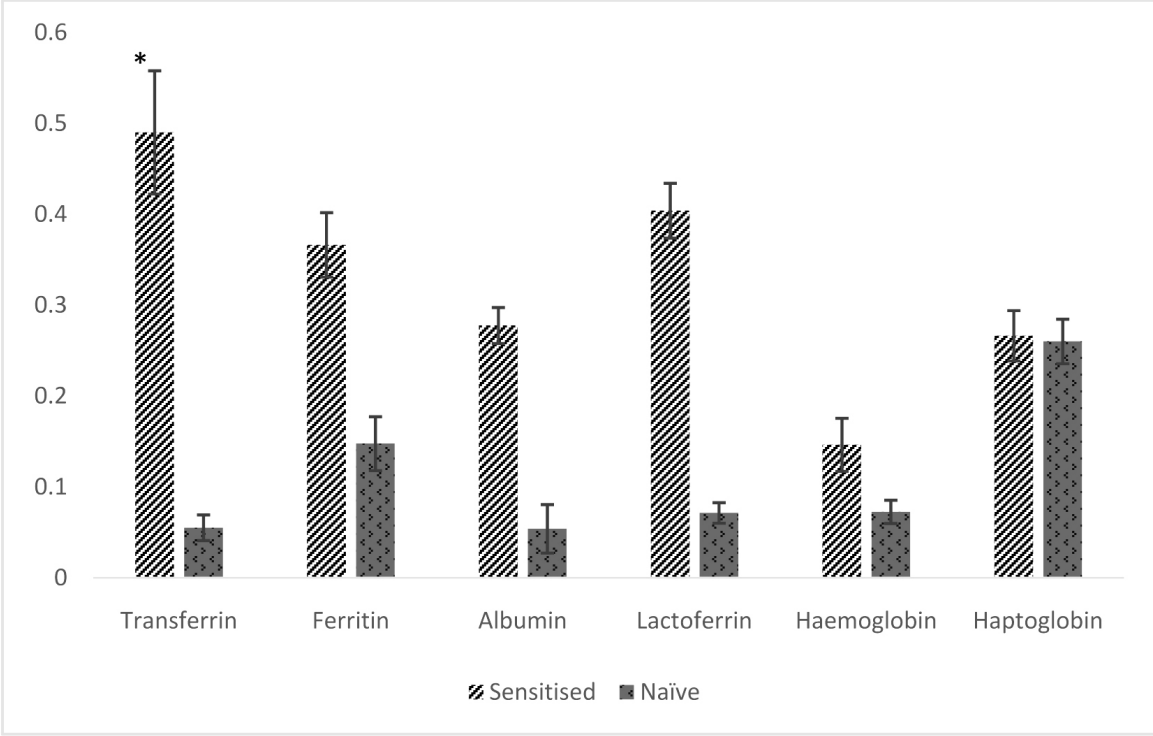


Figure 1. Bar chart showing differences between the means (±S.D.) of the $OD_{450}$ of the immunoreactivity of IgG to the iron-binding proteins.

The ANOVA comparison of the means of each of the iron-binding proteins in mange-positive and naïve pigs indicates that only transferrin was shown to be statistically significant ($p < 0.05$). Ferritin and albumin had no $p$-values while lactoferrin, hemoglobin, and haptoglobin were all statistically insignificant (Table 2).

The membranes for the western blot analysis for the immunoreactivity of the naïve and mange-positive pigs to the iron-binding proteins showed that there was no observed color output with purified Ig obtained from naïve pigs and, also, no IgG antibodies eliciting/reacting with serum proteins from naïve pigs exposed to all the iron-binding proteins used. Mange-positive pigs, however, responded by eliciting IgG-binding antibodies to (ii) bovine holo-transferrin, (iii) bovine apo-transferrin, and (vi) BSA only (Fig.2).

Table 2.One-Way ANOVA Comparison of Means between $OD_{450}$ of Iron-Binding Proteins in Mange-Positive and Naïve Pigs

| Iron-Binding Proteins | Sum of Squares | *F* | *p*-value |
|---|---|---|---|
| Transferrin | 0.032 | 17.592 | 0.020* |
| Ferritin | 0.009 | — | — |
| Albumin | 0.003 | — | — |
| Lactoferrin | 0.005 | 17.792 | 0.180 |
| Haemoglobin | 0.006 | 0.080 | 0.968 |
| Haptoglobin | 0.006 | 0.865 | 0.529 |

*Significant at p<0.05

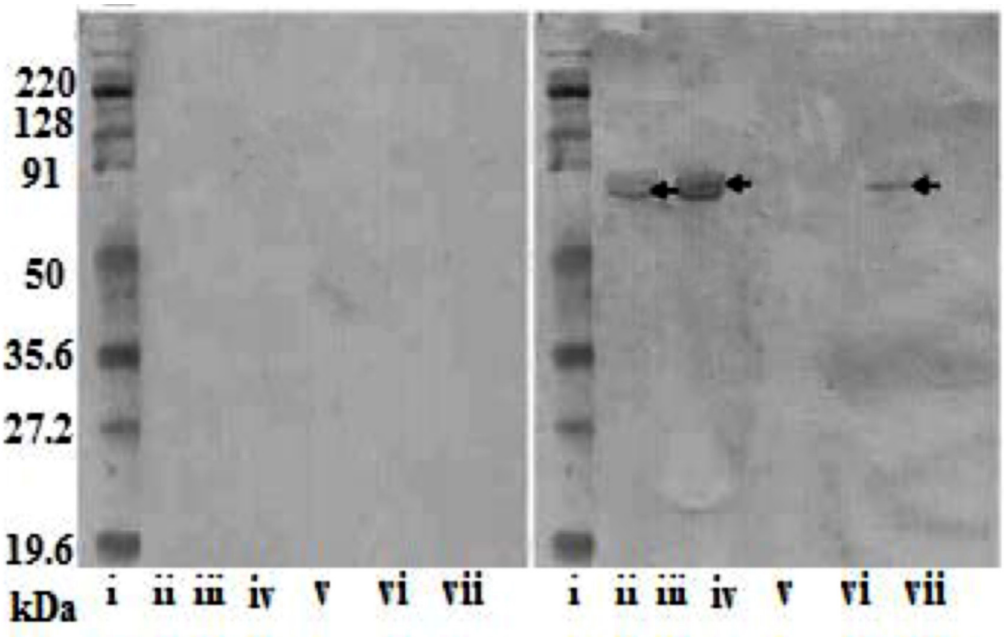


Figure 2.Western blotting of IgG with rabbit anti-pig HRP binding to (ii) bovine holo-transferrin,(iii) bovine apo-transferrin,(iv) dog transferrin,(v) bovine lactoferrin,(vi) bovine serum albumin,and (vii) horse ferritin. (i) represents protein standards. A – naïve pigs; B – mange-positive pigs.

The prevalence of mange in the farms in Ogbomosho was much higher than results obtained by Laha et al. (2014), with the author's survey reporting a lower prevalence in suspected pigs. The author, however, recorded a higher prevalence in organized farms. The higher prevalence in organized farms is probably due to the fact that there is a higher level of contact between the animals kept in pens and enclosures. Melancon (2001) established that the spread of mite between pigs occurs by close contact between the animals or with recently contaminated surfaces. Contact is also reported as the primary route of transmission between animals and humans (Pence & Ueckermann, 2002; Walton & Currie, 2007). Mange infection in pigs is not dependent on growth stage;therefore, age is not a factor in the distribution of scabies. This is contrary to the study of Kagira et al. (2013),where they reported mange infestations being more common in sows, finishers, and growers and least in piglets. The analysis by ELISA revealed that mange-infected pigs produce IgG autoantibodies to transferrin alone. This is in line with reports by Toet et al. (2014) and Zalunardo et al. (2006). However, Toet et al. (2014) also reported a statistically significant difference in IgG autoantibody response to ferritin and transferrin ($<0.05$), while in our study transferrin showed mean difference to $OD_{450}$ and was statistically significant ($<0.05$).Western blot analysis revealed visible antibody responses to bovine holo-transferrin, bovine apo-transferrin, and BSA to rabbit anti-pig HRP. The same responses were observed by Toet et al. (2014). This indicates that iron bound by transferrin and other iron-binding proteins can be sequestered in protein-antibody complexes. This mechanism limits the iron available to the mites and can thus aid in restricting the increase in mite burden on the animals. However, local effects at the site of infection, such as regulation of immune function,cannot be disregarded (De Sousa et al., 1988). The ELISA analysis and western blotting of sera from the mange-positive pigs a reindicative of seroconversion caused by *Sarcoptes scabiei* var. *suis* infestation(Bornstein & Zakrisson, 1993; Bornstein et al., 1995; Hollanders et al., 1997). Diagnosis by monitoring Ig levels in the sera of infected pigs could serve as a good diagnostic tool, although it has been reported in the literature that clinical signs occur before seroconversion (Van der Heijden et al., 2000). Thus, it will be a better diagnostic tool on a herd level as compared to individual tests since control strategies are implemented on both infected and uninfected animals (Jacobson et al., 1999).

## CONCLUSION

The body-to-body contact between the pigs in confined pens as indicated in this study increases the chances of scabies infestation despite antiscabies drug administration since bodily contact is the main route of transmission. Prevalence was, however, reported to be independent of the age of the animal. Physical identification of mites in mange-infected pigs remains the routine but comes along with attendant difficulty, although in the literature it is said to be the most veritable means of diagnosis due to its high sensitivity but low specificity. In this study, IgGwas shown to elicit immunological responses to various *Sarcoptes scabiei* antigens in pigs by reacting with iron-binding proteins indicative of seroconversion caused by *S. scabiei* var. *suis*. Investigation into the use of other immunoglobulins levels in pig sera may also serve as candidate serodiagnostic technique and thus provide in-depth and exciting revelations to ease laboratory management of mange identification in the near future among pig populations.

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### Conflict of Interest

We declare that all the authors agreed to the concept and carried out the study. There is however, no conflict of interest.

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