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Autoantibodies to iron-binding proteins in pigs infested with *Sarcoptes scabiei*

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Abstract

Despite the availability of effective treatments, *Sarcoptes scabiei* remains a major health problem in the pig industry. Unsuccessful control of the disease is often due to the lack of reliable detection methods, with current tests relying on skin scrapings and crude antigen ELISAs. A previous analysis of antigens in pig skin scrapings reported that anti-transferrin antibodies were present in *S. scabiei* infected animals and that this finding might be considered as a useful diagnostic tool. This paper confirms IgG autoantibodies against transferrin, including the first report of IgM autoantibodies, in both naturally and experimentally infected pigs using ELISA and dot blot assays. Autoantibodies were also detected in pigs to ferritin and to a lesser extent lactoferrin. Immunoblotting confirmed the presence of IgG and IgM autoantibodies in mange positive pigs, as well as IgM antibodies to transferrin and albumin in mange negative pigs. These findings suggest the presence of natural autoantibodies to transferrin and albumin in pigs. The development of the IgG autoimmune response may either be a host mechanism for limiting iron to the mite via antibody mediated clearance, the result of host exposure to mite iron-binding homologues or because of a mite-induced antigenic change to host transferrin. Further investigation into the formation of these autoantibodies may provide insights into the importance of iron in scabies infections and the development and perseverance of *S. scabiei* infections in pigs. The specificity and sensitivity of the anti-transferrin response reinforces its potential in the diagnosis of scabies in pigs.

Keywords: Mange; transferrin; ferritin; albumin; lactoferrin; autoantibodies.

Introduction

Sarcoptes scabiei is a parasitic mite responsible for the highly infectious skin disease known as mange or scabies in animals and humans (Mellanby, 1941). This ectoparasite can cause severe skin disorders in over 40 mammalian species, including humans, domestic animals and wildlife (Fain, 1978). Commonly infected species include pigs (Alonso de Vega et al., 1998; Cargill et al., 1997; Davies et al., 1991; Davis and Moon, 1990), dogs (Arlian et al., 1995; Bond, 1998; Feather et al., 2010), foxes (Balestrieri et al., 2006; Newman et al., 2002; Soulsbury et al., 2007), wombats (Martin et al., 1998; Morrison et al., 2003; Skerratt et al., 1998) and humans (Kemp et al., 2002; Walton et al., 2008; Walton and Currie, 2007; Walton et al., 2004).

Sarcoptic mange in pigs, although classed by most veterinarians as controllable, has significant impacts on intensive pig production (Davis and Moon, 1990). Despite the availability of effective acaricides, mange continues to cause economic losses to farmers (Bornstein and Wallgren, 1997; Hollanders et al., 1997; Vercruysse et al., 2006; Wallgren and Bornstein, 1997) and is found at a prevalence of 50-95% worldwide (Alonso de Vega et al., 1998; Cargill et al., 1997). The high incidence of mange in many countries suggests that producers are able to cope with the existence of mange in their piggeries and the associated financial losses (Cargill et al., 1997). As public concern for animal welfare increasingly impacts on animal production systems (Fraser, 2008), the introduction of more stringent animal health guidelines may lead to renewed interest in eliminating sarcoptic mange in pig production.

A major hindrance in the eradication of the disease in both animals and humans is the lack of sensitive diagnostic tests to reliably inform effective management programs (Walton and Currie, 2007). Monitoring antibody levels in individuals by ELISA can be a specific and sensitive method to detect responses to an infection that may be sub-clinical or difficult to diagnose. However, commercial serological assays for the detection of *S. scabiei* use crude mite homogenates from pig (var. *suis*) (Bornstein and Zakrisson, 1993) or fox mite varieties (var. *vulpes*) (Hollanders et al., 1997; van der Heijden et al., 2000) as the antigen source. Mites cannot be easily maintained away from their host for long periods of time (Arlian et al., 1984) and thus antigen is only available in limited amounts for commercial application. As a result, current commercial ELISAs are expensive and detect a plethora of antibody responses with highly variable specificities (Kessler et al., 2003; Löwenstein et al., 2004; van der Heijden et al., 2000). The use of a purified antigen would improve the sensitivity of the assay and should reduce cross-reactivity with other mite species (Hollanders et al., 1997). A further improvement to these assays would be the ability to produce recombinant antigen in sufficient quantities and at a cost suitable for widespread usage.

Previous research by Zalunardo *et al.* (2006) analysed a skin scraping extract from *S. scabiei* infected pigs as a source of mite antigens for diagnosis. Immunoblotting with mange positive sera identified a number of specific antigens in these extracts. An antigenic protein of 80 kDa was determined by N-terminal sequencing to be pig transferrin. In addition, mange positive dog IgG when coupled to a CNBr-activated sepharose affinity column bound a 40 kDa host protein with autoantigenic properties identified as haptoglobin. High antibody titres were detected by ELISA in mange positive pigs against both transferrin and haptoglobin. The suggestion was made that the presence of these autoantigens may be a diagnostic tool for the detection of mange in pigs (Zalunardo et al., 2006). The present study aimed to confirm the

initial findings of host autoantibodies induced by mite infections in pigs, investigate the source of the autoantibodies and the dynamics of their production during infection with and without immunosuppressive treatment.

2. Materials and Methods

2.1 Source and collection of animal sera

The collection of sera for this research was approved by the La Trobe Animal Ethics Committee, La Trobe University, Bundoora, Victoria, Australia.

Sera from pigs experimentally infected with *S. scabiei* were obtained from the Queensland Institute of Medical Research (QIMR, Royal Brisbane Hospital, Brisbane, Queensland, Australia). Experimental infections, pig housing and mite sampling is described in Mounsey *et al.* (2010). Pigs were allocated into two treatment groups: mange negative (n = 2) and mange positive (n = 4). Blood was taken at 0, 2, 4, 8, 12 weeks post infection and the serum fraction collected. Over the 12 week period, clinical development of scabies was monitored by measuring skin scores (score of 1-8, 8 being most severe), and skin scrapings for collected for mite counts. Pig experimental infections were conducted with ethical approval from both DEEDI and QIMR Animal Ethics Committees.

Additional sera from naturally infected gilt sows (5-6 months old, n = 20) and uninfected 15 week old pigs (n = 20) were sourced from commercial piggeries in Victoria with the assistance of Dr Patrick Daniel of the Pig Health Research Unit, Department of Primary Industries (DPI), Bendigo, Victoria, Australia. Animals were confirmed positive for mange

based on the identification of direct symptoms diagnostic of mange, such as papules or the presence of mites, by the Extension Officers on farm and at slaughter.

2.2 Indirect enzyme-linked immunosorbent assay (ELISA)

The ELISA described here was optimised by a checkerboard titration using confirmed mange positive and negative pigs and rabbit anti-pig IgG-HRP antisera (Sigma, Saint Louis, Missouri, USA). Commercially produced serum proteins were diluted to a concentration of 10 µg/ml with carbonate buffer (0.015 M Na_2CO_3 and 0.035 M $NaHCO_3$, pH 9.6). Antigens tested included transferrin (bovine, human or dog), haptoglobin (human), ferritin (horse spleen), hemoglobin (bovine), albumin (Bovine Serum Albumin; BSA) and lactoferrin (bovine) (Sigma). Each well of a 96 well ELISA microplate (U-bottom MICROLON®, medium binding; Greiner Bio-one, Germany) was coated with 50 µl of an antigen solution and plates were incubated at 4 ºC overnight. Plates were washed three times with phosphate buffered saline containing Tween 20 (PBS-T; 16 mM Na_2HPO_4, 5 mM NaH_2PO_4, 120 Mm NaCl, 0.05 % (v/v) Tween 20, pH 7.4) between each step. Plates were blocked for 2 h at room temperature (RT) with 200 µl of PBS-T containing 3% (w/v) BSA, except for assays testing for antibodies to albumin where 5% (w/v) skim milk was used in place of BSA. Serum was diluted 1/200 in PBS-T containing the appropriate blocking agent and incubated at RT. Each sera was tested in triplicate on each plate. Plates were incubated at 37 ºC for 1 h. Secondary antibodies (rabbit anti-pig IgG-horseradish peroxidise (-HRP) antibody, Sigma, and goat anti-pig IgM-HRP antibody, Bethyl Laboratories, Inc., Montgomery, TX, USA) were diluted to 1/2000 in PBS-T containing the appropriate blocking agent and incubated at RT for 30 min with shaking. A volume of 50 µl was added to each well and incubated for 1 h at 37 ºC. Plates were washed three times for 15 min with PBS-T and a final wash with dH_20 for 15 min. The substrate 3,3',5,5'-Tetramethylbenzidine (TMB; Invitrogen, CA, USA) was

added at a volume of 50 µl to each well and incubated in the dark at RT for 15 min and then 50 µl of 1 M HCl was added per well. Plates were read after 5 min at an absorbance of 450 nm on a microplate reader.

The cut-off values between negative and positive results were calculated as the average result of the mange negative animals plus three times the standard deviation (S.D.).

The sensitivity and specificity for each assay were calculated using the following formulas:

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{True positives + false negatives}} \times 100\%$$

$$\text{Specificity} = \frac{\text{True negatives}}{\text{True negatives + false positives}} \times 100\%$$

The statistical significance between the positive and negative mange animals for each protein tested was determined using a Mann-Whitney U-test using Minitab® Statistical Software Version 14 (Minitab, Sydney, Australia).

2.3 Isolation and purification of pig immunoglobulins by protein A column

Natural infection and uninfected pig serum aliquots were combined to form two larger pools, respectively, of 500 µl and prepared for Ig purification by mixing with equal volumes (1:1) of degassed Tris HCl running buffer (0.1 M Tris, 0.135 M NaCl, pH 8.0). Diluted serum was loaded onto a Protein A affinity column (protein A-Sepharose; Invitrogen) and running buffer was added until flow through was complete. Bound Ig was eluted with degassed 0.1 M

glycine elution buffer, pH 2.7, and the eluted Ig fractions were collected in tubes and 10% (v/v) of Tris collection buffer (1 M Tris, pH 8.0) was added to neutralise pH. Ig was stored at -80 °C before freeze drying in an Ilshin Freeze Drier (Model TFD8503, Ilshin Lab Co. Ltd., Europe). Samples of freeze-dried purified Ig were passed through the Sephadex G-25 column (Amersham Biosciences, Uppsala, Sweden) with degassed PBS-T buffer. Concentration of purified Ig was calculated using a bicinchoninic acid (BCA) protein estimation kit (Pierce® BCA Protein Assay; Pierce Biotechnology, Rockford, IL, USA).

2.4 Western blot analysis of serum proteins with protein A purified immunoglobulins

Commercial lyophilised serum proteins (transferrin, albumin, ferritin) were dissolved in dH_2O and approximately 5 µg of each protein was added to 10 µl of 2x sample buffer (0.5 M Tris-HCl, pH 7.0, 5% w/v SDS, 120 mM dithiothreitol (DTT), 0.5% w/v bromophenol blue and 20% v/v glycerol). Sample preparation and electrophoresis was performed according to the modified method by Laemmli (1970). After electrophoresis, gels were equilibrated in transfer buffer (0.3% w/v Tris, 1.5% (w/v) glycine, 20% (w/v) methanol in dH_2O for 15 min prior to transfer to Polyvinylidine difluoride (PVDF) Immobilon-P transfer membrane (Millipore, Bedford, U.S.A). Electro-transfer was conducted using the modified method of Towbin *et al.* (1979). After transfer, the membranes were washed once with PBS-T and then blocked with 5% (w/v) skim milk powder in PBS-T overnight at 4 °C. Membranes were washed three times with PBS-T. Purified pig antibody (1 mg/ml) was diluted 1/100 in PBS-T and incubated for 1 h. Membranes were washed three times in PBS-T for 5 min and incubated in rabbit anti-pig IgG-HRP (Sigma) or goat anti-pig IgM-HRP (Sigma) diluted 1/5000 in PBS-T for 1 h at RT. After incubation, the membranes were rinsed in PBS-T three times for 15 min each wash and then washed once in dH_2O. Membranes were developed using a 3',3'-Diaminobenzidine (DAB) substrate kit including metal enhancers (Sigma).

200

201 **2.5 Dot blot analysis**

202 PVDF membrane was divided into 2 cm^2 grids. In the centre of a single grid, 2 µl of 10 µg/ml
203 transferrin was spotted and allowed to dry at RT. Strips were blocked with 5% (w/v) skim
204 milk in PBS-T for 2 h at RT. Strips were washed three times with PBS-T. Individual naturally
205 infected and negative pig serum samples diluted 1/10 in PBS-T were added to each spot at a
206 volume of 10 µl. The strips were covered and kept at RT for 1 h, then washed three times in
207 PBS-T. Strips were incubated at RT with secondary antibody, rabbit anti-pig IgG-HRP
208 diluted 1/5000 in PBS-T for 1 h. Strips were then washed three times for 15 min with PBS-T
209 and then a final wash with dH_2O. Strips were developed with DAB as described then allowed
210 to dry. Strips were analysed by calculating the mean integrated density of the probed dot
211 using Image J analysis software Version 1.44n (National Institutes of Health, USA).
212 Differences of the integrated density results for positive versus negative mange pigs were
213 determined by using a Mann-Whitney U-test (Minitab® Version 14; Minitab).

214

215

216 **3. Results**

217 **3.1 Analysis of IgG & IgM immunoreactivity of pigs to serum proteins by ELISA**

218 Higher IgG antibody levels to transferrin, lactoferrin and ferritin in mange positive pigs were
219 recorded, but not for albumin, haptoglobin or hemoglobin (Fig. 1). The average IgG
220 responsiveness to transferrin was significantly lower for the mange negative pigs compared to
221 the mange positive pigs (Mann-Whitney U-test, $P<0.05$). A cut off value was determined
222 from the average OD_{450} value of the mange negative pigs + 3 S.D., of 0.109. The OD_{450} of all
223 positive pigs was greater than the calculated cut off value and no negative pigs displayed

OD_{450} values greater than 0.109, giving this assay a diagnostic specificity and sensitivity of 100% . For ferritin, the mean OD_{450} of the negative pigs measured was significantly lower than the mean response of positive pigs (Mann-Whitney U-test, $P<0.05$). The cut off value was calculated as 0.195. Based on this, the specificity of the assay was 100% with a sensitivity of 90%. On average, mange positive pigs produced a higher OD_{450} reading than that of negative pigs to lactoferrin, however this difference was not statistically significant (Mann-Whitney U-test, $P>0.05$). A cut off value was not applied to this data.

On average, mange positive pigs displayed higher IgM responsiveness to transferrin (OD_{450}) than that of the negative pigs (Fig. 2). A cut off value was determined at 0.768, which resulted in a specificity of 90% and a sensitivity of 50%. For albumin, there was little difference between the mean OD_{450} of the positive pigs compared with that of the negative pigs. Mann-Whitney U-tests were performed to determine if the difference in the average IgM OD_{450} of positive pigs compared with that of negative pigs was significant. A significant difference was found for the transferrin assay (Mann-Whitney U-test, $P<0.05$), but not for albumin (Mann-Whitney U-test, $P>0.05$).

3.2 Testing of pig anti-transferrin antibodies during an experimental infection with *S. scabiei*

In an experimental infection model of pigs with *S. scabiei*, pigs displayed common symptoms of acute-hypersensitive porcine mange, such as a papular rash, hair loss, reddened skin, flaking scabs and scratching behaviour. Infections, however, were generally short-term, with skin scores peaking between weeks 8 and 10 post infection before reducing. This acute-term infection provided low mite recovery and gave a clinical condition similar to ordinary scabies

248 in humans. In two pigs, clinical conditions more closely resembled crusted scabies with high
249 mite counts observed.
250
251 On average, mange positive pigs displayed greater transferrin reactivity over the infection
252 program at weeks 8 and 12 (Fig. 3). Despite a low coefficient of determination value (R^2 =
253 0.362), a significant relationship was determined using the linear regression equation OD =
254 0.301 + 0.00114 Mites (ANOVA F-test, $p<0.05$). Therefore it was determined that mite
255 burden was a poor predictor of the OD value obtained from the transferrin ELISA. This was
256 evident when comparing individual pig anti-transferrin responses at the different time points.
257 For example, pig 4162B had the highest antibody response at 12 weeks (mean OD_{450} 1.029)
258 with only ten mites recovered from skin scrapings. High numbers of mites (500 mites) were
259 counted from skin scrapings conducted at 12 weeks for pig 4160B, however the
260 corresponding antibody responses (mean OD_{450} 0.693) was moderate compared to pig
261 4162B.
262

263 **3.3 Western blotting of commercial serum proteins probed with purified pig**
264 **immunoglobulins**

265 No reactivity to transferrin was observed with pooled Ig purified from mange negative pigs
266 tested by ELISA. Blots probed with sera from mange negative pigs and anti-pig IgM showed
267 very little staining of bands (Fig. 4A), with the strongest bands identified being bovine
268 transferrin (*holo*- and *apo*-). Mange positive pig IgM showed strongest antibody binding to
269 bovine *holo*- and *apo*-transferrin (Fig. 4B). Little binding was observed by mange positive
270 pig IgM antibodies to lactoferrin and dog transferrin. Mange positive IgM binding was also
271 observed for bovine serum albumin. No IgG antibodies to serum proteins were detected in the
272 purified immunoglobulins from mange negative pigs (Fig. 4C). Mange positive pigs had IgG

antibodies to bovine *holo*- and *apo*-transferrin and bovine serum albumin (Fig. 4D). Mange positive pig IgG also displayed affinity for dog transferrin, bovine lactoferrin and horse ferritin (Fig. 4D).

3.4 Dot blot analysis of anti-transferrin antibodies

An investigation was carried out to determine the efficiency of a developed dot blot assay to detect pig anti-transferrin IgG antibodies and whether this assay would show differential binding to the ELISA and be useful as a diagnostic tool. The colour intensity (integrated density) visualised from the dot blot was greater for most of the positive pigs (n = 10) compared with the negative pig blots (n = 10) (Fig. 5). The dot blot was analysed by calculating the mean integrated density for each individual sample using Image J Software. Positive pigs had an average of 20.3 ± 17.4 integrated density units (IDU) and the mean value for the mange negative pigs was calculated as 6.9 ± 3.2 IDU. The mange positive pigs had a statistically higher mean than the uninfected pigs (Mann-Whitney U-test, $P < 0.05$). This gave the assay a specificity of 90% and a sensitivity of 80%. To determine if the dot blot assay could be used as a diagnostic assay for the detection of mange in pigs, the mean integrated density values determined for each individual pig were compared with the corresponding OD_{450} values obtained from the single dilution indirect ELISA testing for antibodies to bovine transferrin. Regression analysis was used to determine the correlation coefficient between the OD_{450} and mean integrated density. A general trend was observed between the two data sets with a R^2 value of 0.887. The calculated equation for this correlation was Dot = 2.407 + 47.11 ELISA. This correlation was considered significant using an ANOVA F-test (ANOVA F-test, $P < 0.05$). Further testing of the transferrin Dot Blot assay gave an average value of 15.09 ± 12.55 IDU for mange positive pigs (n = 20) and the mean value for the mange negative pigs (n = 20) was calculated as 3.35 ± 2.98 IDU. The mange positive pigs had statistically higher

values on average than the mange negative pigs (Mann-Whitney U-test, $P < 0.05$). A cut off based on these results using the mean of the mange negative pigs ± 2 S.D. was determined as 9.3 IDU. This gave the assay a specificity of 95% and sensitivity of 70%.

4. Discussion

Analysis by ELISA, western blotting and dot blots confirmed the report by Zalunardo *et al.* (2006) that mange positive pigs produce IgG autoantibodies to transferrin. Mange positive pigs did show an autoantigenic IgG response to lactoferrin and ferritin, both of which are also iron-binding proteins. Transferrin and lactoferrin belong to the same family of monomeric iron-binding proteins, the transferrins, and share similar structure and functions (Aisen and Brown, 1975; Farnaud and Evans, 2003; Nichol et al., 2002). This suggests that an autoimmune response targeting iron-binding proteins may have some relation to their function in the host.

The experimental infection of mange conducted by scabies researchers at QIMR provided an opportunity to observe the development of transferrin autoantibodies over the course of infection with *S. scabiei*. In three out of four pigs experimentally infected, antibody reactivity was observed to rapidly increase between weeks four and eight. Antibodies to *S. scabiei* are detectable as early as four weeks post infection (Kessler et al., 2003; Wooten and Gaafar, 1984), with complete seroconversion in most individuals detectable at six weeks (Bornstein and Zakrisson, 1993; Bornstein et al., 1995; Kessler et al., 2003; Nöckler et al., 1992; van der Heijden et al., 2000; Wooten and Gaafar, 1984).

Serum IgM antibodies to transferrin were identified in mange positive pigs displaying anti-transferrin IgG antibodies. Low level IgM antibodies to transferrin were also detected in mange negative pigs that did not produce anti-transferrin IgG. IgM autoantibodies (known as natural autoantibodies) to transferrin have been well documented in humans (Avrameas et al., 1981; Guilbert et al., 1982; Seigneurin et al., 1988; Vassilev and Veleva, 1996) and are naturally present in healthy individuals (Avrameas et al., 1981; Boyden, 1965). It has been established that the autoantigenic repertoire is selected in early neonatal development. Adult autoreactive B cells may be further selected as a result of their individual immunological exposure (Meffre and Salmon, 2007; Merbl et al., 2007). Autoreactive B cells secreting IgM require stimulation by selective autoreactive T cells for the class switch to IgG autoantibody production. Age, environmental exposure (stimulation) and genetics may all play a role in the selection of specific autoreactive B cells and T cells, thus dictating the autoantigens detectable in an individual (Merbl et al., 2007). The presence and preservation of natural autoantibodies indicates that they may play a role in the animal's immune repertoire. Natural autoantibodies to transferrin may be involved in regulation of transferrin by clearing the body of proteins whose function has been altered (Guilbert et al., 1982), supporting the theory presented by Zalunardo *et al.* (2006).

The confirmation that pigs develop anti-transferrin antibodies would indicate that iron bound by transferrin could be sequestered in transferrin-antibody complexes and thus possibly restricted from mites. If an iron-sequestering mechanism does occur then its ability to limit mite burdens depends on more than the immune responsiveness of individual pigs. Tests for free iron in the serum of pigs may indicate whether the effect of the transferrin autoantibody would be limiting free iron concentration, at least systemically, although local effects at the site of infection could not be disregarded.

A third mechanism for autoantibody production would suggest that *S. scabiei* transferrin molecules share antigenic determinants with pig transferrin. The presence of mites within the skin exposes the host to mite transferrin homologues. If shared epitopes exist between the host and mite proteins, this may stimulate the production of autoantibodies due to antigenic cross-reactivity (Cojocaru et al., 2009). As *S. scabiei* burrows within the skin, dead mites deteriorate within the epidermis and host immune components will encounter various mite proteins. This results in the host producing antibodies to new antigens and a stronger immune response (Arlian and Morgan, 2000). As shown in this paper, anti-transferrin IgM autoantibodies are already present in the pig host. As a result, the exposure to antigens with shared or similar epitopes may provoke a stronger, more specific IgG autoimmune response that also targets the host protein.

A dot blot assay was developed to determine its efficacy in detecting transferrin autoantibodies in pigs. The described assay identified mange positive pigs based on the production of autoantibodies to transferrin, although it was not as sensitive as the ELISA. This method did not detect autoreactive IgG antibodies in uninfected pigs, consistent with the ELISA data. The described assay may be useful as a faster and more cost effective method for identifying mange positive animals within a herd and would have potential as a farm-based assay. They are also favourable assays for the detection of autoantibodies (Ghirardello et al., 2009).

5. Conclusion

In this paper, transferrin was confirmed as an autoantigen in pigs. Another iron-binding protein, ferritin, was found to be a significant autoantigen in pigs infected with *S. scabiei*.

Research into the mechanism for the formation of autoantibodies in mange positive pigs is needed to determine if it is the result of cross-reactivity with *S. scabiei* proteins or the result of infection induced changes to the host protein.

Conflict of interest statement

The authors have no conflict of interest.

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Figure captions

Fig. 1. ELISA of the IgG immunoreactivity of pigs to various self proteins. Pig sera tested were from a confirmed mange positive piggery (n = 20) and a confirmed mange negative piggery (n = 20). Values given are the mean OD_{450} value for each group of pigs ± S.D. Asterisk (*) indicates significant difference ($P< 0.05$) observed between positive and negative pigs.

Fig. 2. ELISA of IgM immunoreactivity of pigs to various self proteins. Pig sera tested were from a confirmed mange positive piggery (n = 20) and a confirmed mange negative piggery (n = 20). Values given are the mean OD_{450} value for each group of pigs ± S.D. Asterisk (*) indicates significant difference ($P< 0.05$) observed between positive and negative pigs.

Fig. 3. Progression of anti-transferrin IgG antibodies in pigs experimentally infected with *S. scabiei*. Group A: mange positive pigs (n = 4), group B: mange negative pigs (n = 2). Error bars represent one standard deviation (1 S.D.).

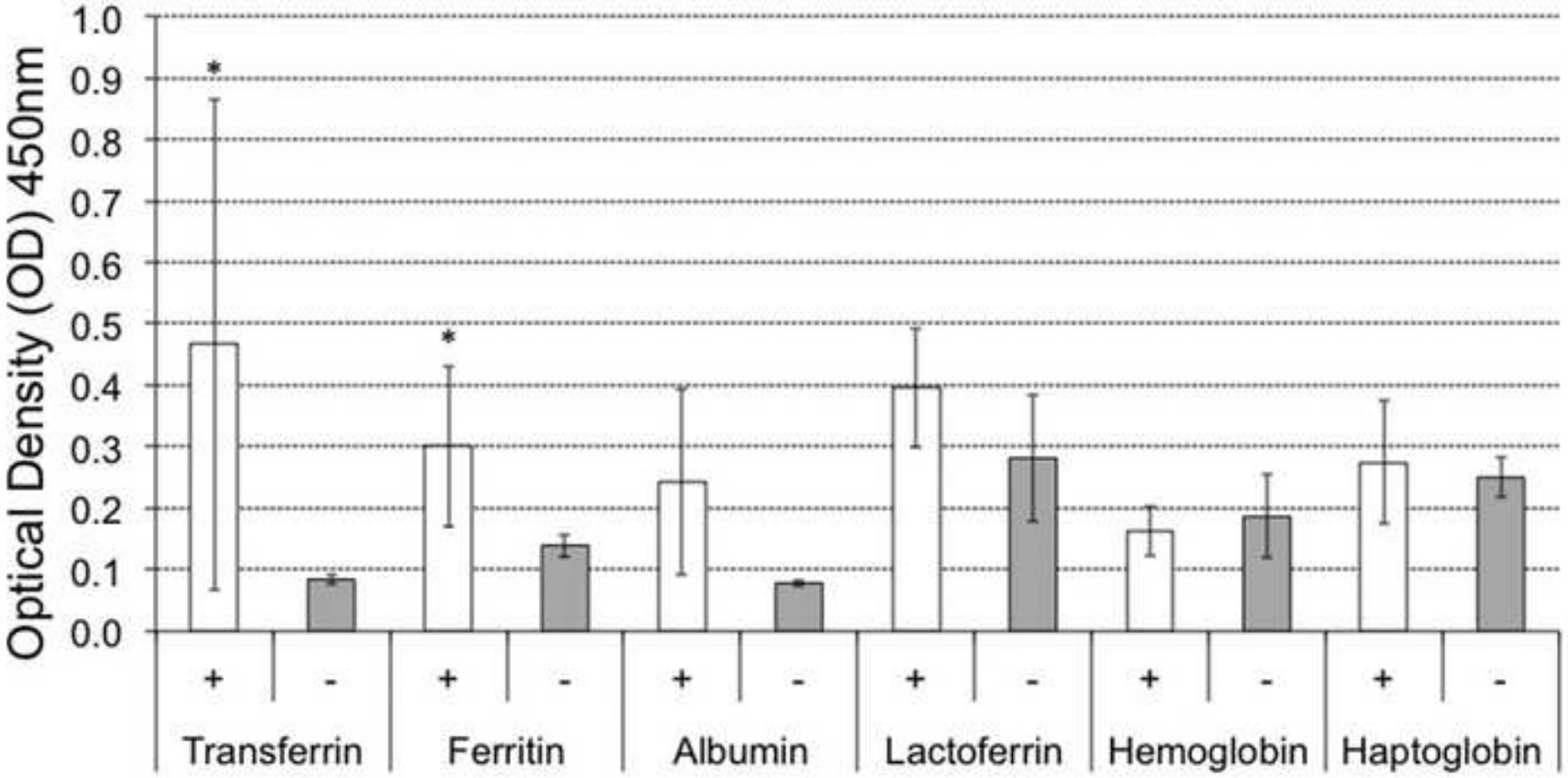
Fig. 4. Western blots of serum proteins probed with mange negative (blots A/C) and mange positive (blots B/D) purified pig immunoglobulins, detected by goat anti-pig IgM-HRP (blots A/B) and rabbit anti-pig IgG-HRP (blots C/D). Lane 1: protein standards, lane 2: bovine *apo*-transferrin, lane 3: bovine *holo*- transferrin, lane 4: dog *apo*-transferrin, lane 5: bovine lactoferrin, lane 6: bovine serum albumin, lane 7: horse spleen ferritin. Arrows indicate bands.

Fig. 5. Dot blot analysis of pig antibodies to bovine transferrin. Mange positive pigs (n = 20) and mange negative pigs (n = 20). Asterisk (*) indicates significant difference ($P< 0.05$) observed between positive and negative pigs.

Highlights

We confirm the development of anti-transferrin IgG in *S. scabiei* infected pigs.
We present the first report of anti-transferrin IgM in *S. scabiei* infected pigs.
These findings suggest natural autoantibodies to transferrin and albumin in pigs.
Transferrin autoantibodies are produced due to mite interaction with the host.
The anti-transferrin ELISA has potential in the diagnosis of scabies in pigs.

Figure 1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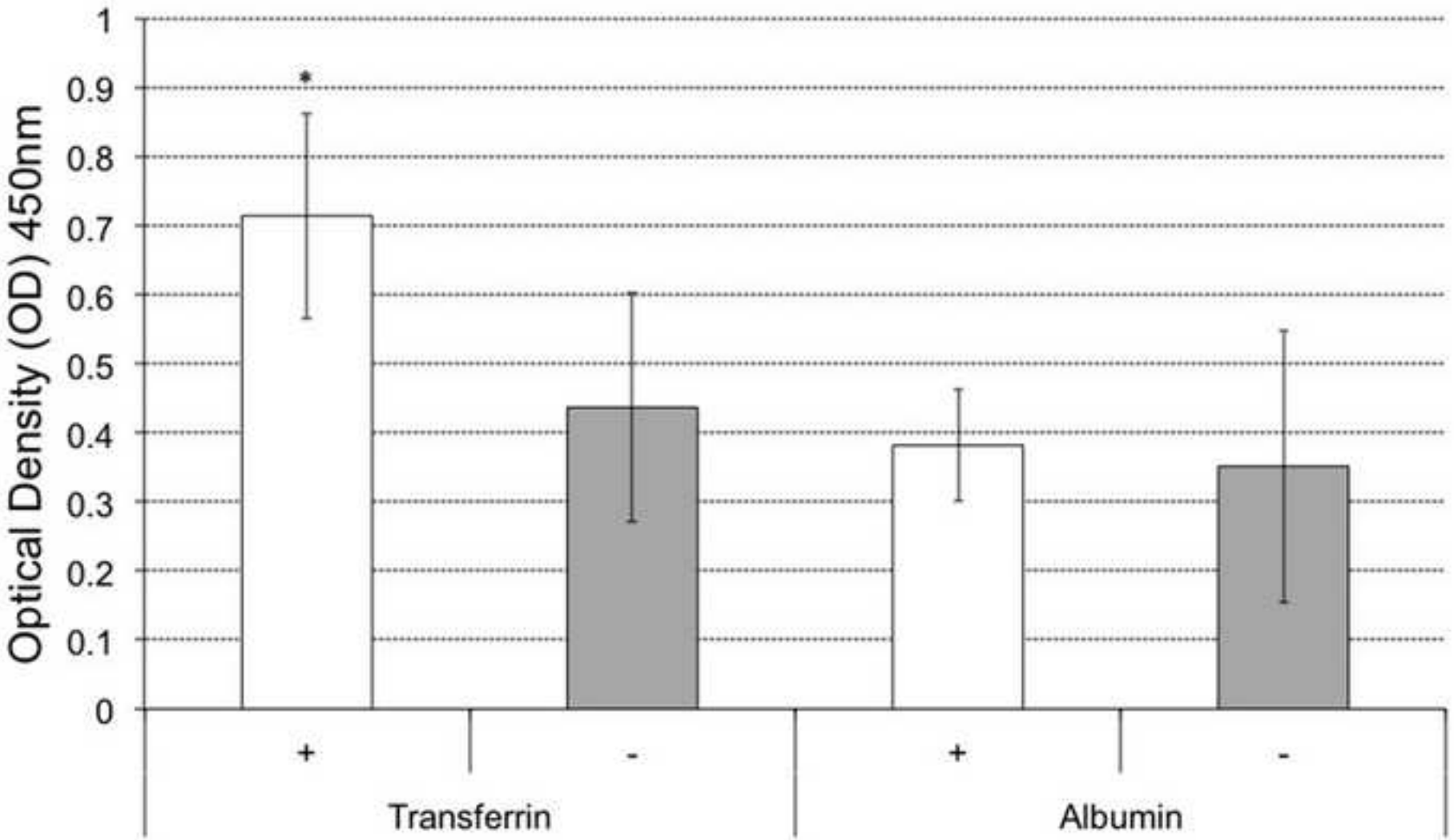
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
*
+
-
+
-
+
-
+
-
+
-
+
-
Transferrin
Ferritin
Albumin
Lactoferrin
Hemoglobin
Haptoglobin

Figure 2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0.8
0.7
0.6
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0.3
0.2
0.1
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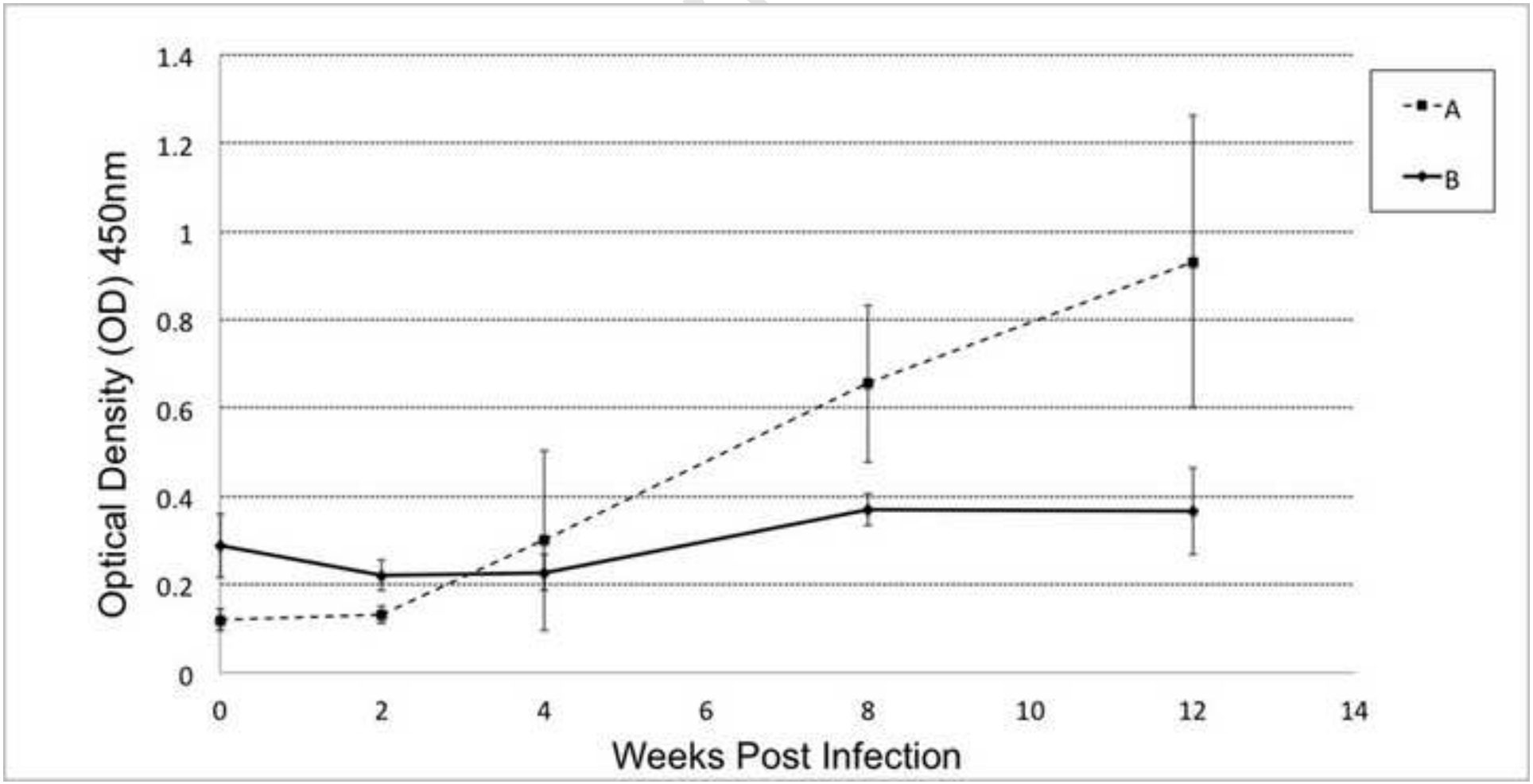
*
+
-
+
-
Transferrin
Albumin

Figure 3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0
0
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4
6
8
10
12
14
Weeks Post Infection
A
B

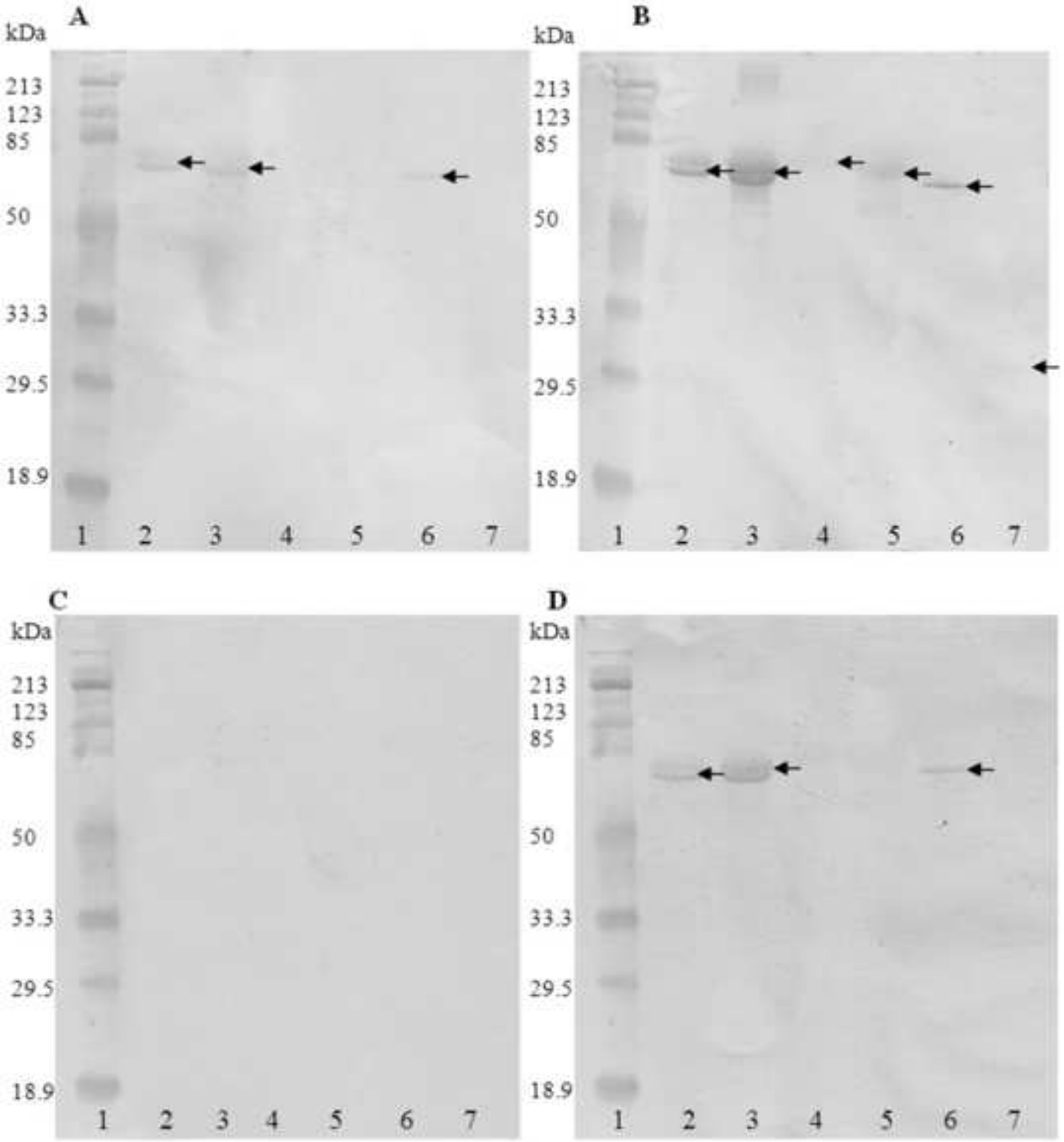
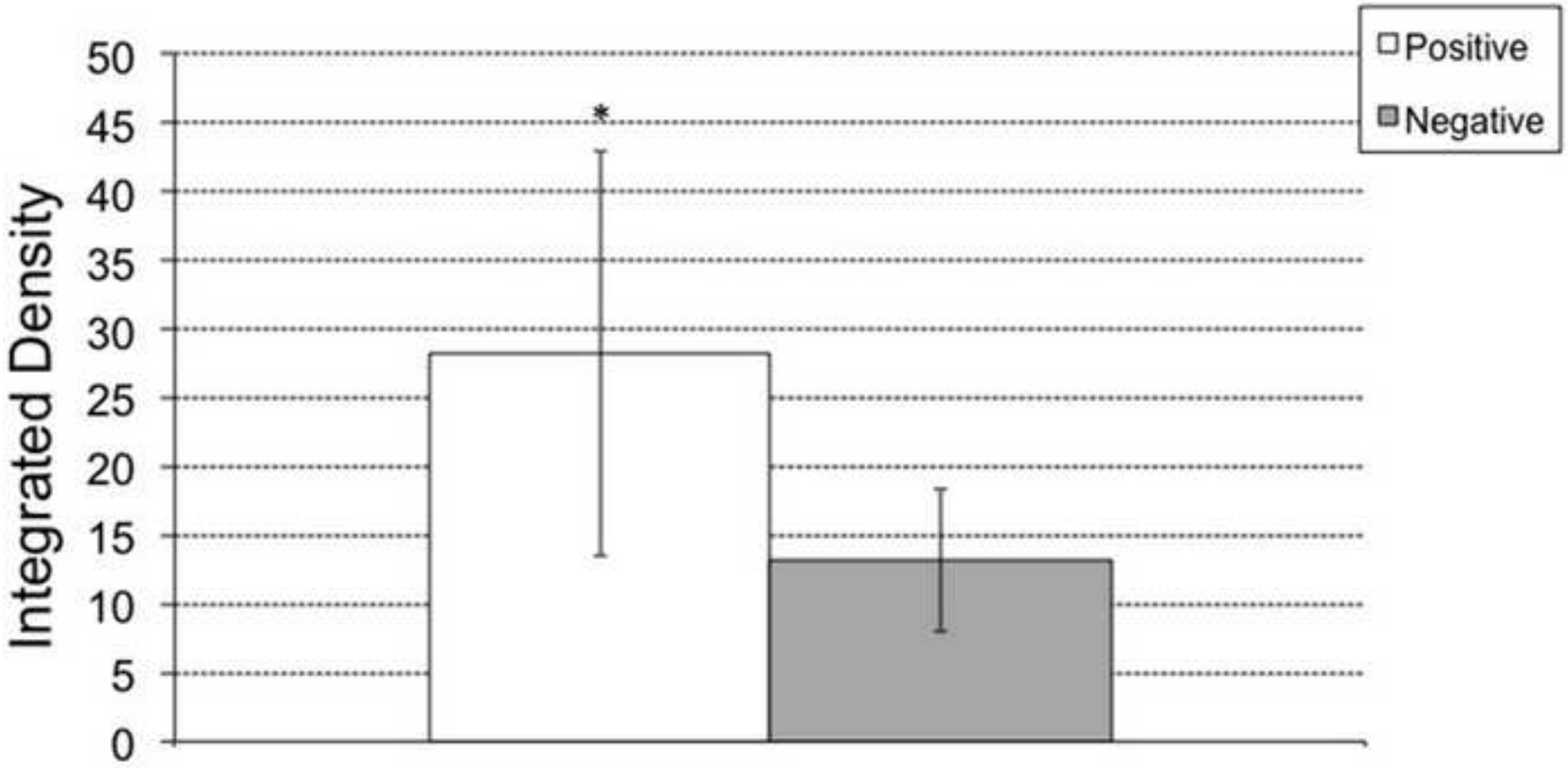


Figure 5


Integrated Density
50
45
40
35
30
25
20
15
10
5
0
*
Positive
Negative