Comparative efficacy of two parenteral iron-containing preparations, iron gleptoferron and iron dextran, for the prevention of anaemia in suckling piglets

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ABSTRACT
Iron-deficiency anaemia (IDA) is a serious health problem in neonatal piglets and is controlled by routine application of iron in various formulations. The efficacy and safety of two iron-containing products for the prevention of IDA in suckling piglets were compared in a randomised, parallel study. Newborn piglets were treated with 200 mg iron supplied by intramuscular injection in the neck as either Forceris (gleptoferron; n=13) or Uniferon 200 (iron dextran; n=12) 24–48 hours after birth. Blood samples were collected before and after treatment (2nd, 18th and 31st day of life) for complete haematology. The treatments were well tolerated with only mild transient swelling observed in two piglets (Forceris group). Piglets treated with Forceris had significantly higher haemoglobin, haematocrit, mean corpuscular volume and haemoglobin concentration values, as well as significantly higher plasma iron and transferritin saturation and a lower total iron binding capacity than those treated with Uniferon. No animals in the Forceris group but 17 per cent of piglets in the Uniferon group had haemoglobin levels <9 g/dl after treatment, indicating anaemia. These results suggest that both products were safe and effective in the prophylaxis of IDA in piglets, and that Forceris was superior to Uniferon in preventing IDA in piglets.

INTRODUCTION
Iron deficiency anaemia (IDA) is the most commonly recognised clinical condition of fast-growing piglets reared under intensive conditions and is considered as an emerging problem in modern swine production.1 IDA develops in piglets which do not receive iron supplementation, which is due to various factors, including the low body iron reserves at birth, the low iron content of the sow’s milk.2 3 The rapid growth rate of the newborn piglet with its high requirement for a large amount of haemoglobin-carrying red blood cells4 and limited access to soil as an iron source.5 6 These factors result in a predictable drop in haemoglobin and other iron-carrying molecules in the piglets’ blood, and IDA inevitably develops unless supplemental bioavailable iron is administered shortly after birth. The primary consequences of IDA are reduced growth rate and increased susceptibility to infectious diseases. Attempts to increase the placental transfer of iron to the fetus or the iron concentration in milk by feeding high levels of iron to sows or parenteral administration of high iron doses were not successful.7 8 The efficacy of oral iron application is hampered by the very low expression of duodenal iron transporters in pigs.9 15 An oral combination of toltrazuril and iron dextran (Baycox Iron; Bayer Animal Health, Monheim, Germany) was recently evaluated compared with intramuscular iron injection, confirming lower haemoglobin levels at 21 days of age in the oral combination-treated piglets.16 In addition, oral iron therapy may be limited by gastrointestinal side effects, such as nausea, vomiting, abdominal pain and diarrhoea.17 18 Iron as iron-carbohydrate complexes for parenteral application is commonly administered to newborn piglets to prevent IDA.1 19 The first synthesis of iron dextran complex for intramuscular use dates back to 1952.20 It regenerates haemoglobin quickly and efficiently in human beings and piglets, and is well tolerated.14 Different preparations containing various iron-carbohydrate complexes were subsequently evaluated for improved safety, bioavailability and efficacy.21 Gleptoferron is a complex of beta-ferric oxyhydroxide and dextran glucoheptonic acid in an aqueous colloidal solution. It has previously been demonstrated that gleptoferron is comparable to iron dextran in the prevention of IDA in piglets.22 23 A more recent study showed...
that gleptoferron treatment resulted in higher plasma iron levels compared with iron dextran. In a new formulation, gleptoferron was combined with the antioccidial toltrazuril in a ready-to-use injectable composition for the simultaneous metaphylaxis of piglet cystoisosporosis (coccidiosis) and IDA prevention. The present study was conducted to evaluate the haematinic efficacy of this new combination product (Forceris) compared with iron dextran (Uniferon 200) in piglets.

MATERIALS AND METHODS
Experimental design and treatment
The study was performed according to a parallel, randomised, blinded experimental block design. It included four sows and their litters in one experimental unit. The experimental unit was the individual piglet.

Samples were taken during a study comparing Forceris with a combination of Uniferon 200 plus oral toltrazuril and a group treated with Uniferon 200 only (not included here) against experimental infections of piglets with Cystoisospora suis. Clinical, parasitological and safety data were published elsewhere.

Pregnant sows (Landrace x Large White) were moved to the large animal facilities of the Institute of Parasitology of the Vetmeduni Vienna two weeks before farrowing to acclimatise to the housing conditions. Animals were kept on straw with a heat lamp for the piglets and fed with conventional feed. Water was provided ad libitum. Within 24 hours after birth (study day 1; SD 1), animals were identified individually and enrolled in the study if they were clinically healthy and weighed at least 900 g. The same day, individual piglets within each litter were randomly allocated to one of two treatment groups according to bodyweight in the order of the birth of the litters.

On SD 2 (24–48 hour after birth), the treatment group received 200 mg of iron/piglet as gleptoferron (Forceris; Ceva Santé Animale, Libourne, France; 30 mg/ml of toltrazuril and 133.4 mg/ml of iron as gleptoferron) and the control group was treated with 200 mg/piglet of iron hydroxide dextran complex (Uniferon; Virbac, Holbaek, Denmark). Both were injected intramuscularly in the neck. To account for the toltrazuril in Forceris, the control animals received 20 mg toltrazuril/kg of bodyweight (Baycox 5%; Bayer Animal Health, Monheim, Germany) on SD 4 as recommended by the manufacturer.

Piglets were weighed on SD 1 and then weekly until the end of the study. The general health of the sows and their piglets was observed and recorded daily from SD 1 to SD 31 (end of study). No creep feed was offered before 14 days of life, but piglets had access to sow’s feed (100 mg/kg of iron (ii) sulfate).

Blood sampling, haematological analysis and bodyweight development
Blood samples (with lithium heparin as anticoagulant) were collected from piglets before treatment (SD 2) and on SD 18 and SD 31 by puncture of the vena cava. Haematological parameters were evaluated by the Central Laboratory, Department of Pathobiology, University of Veterinary Medicine Vienna: erythrocyte count (Erys), haemoglobin level (Hb), haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using a laser-assisted automated analyser (AVIDA 2120, Siemens Healthineers, Vienna, Austria). In case of large numerical or graphical deviations, stained individual blood smears were examined additionally. Plasma iron concentration and total iron binding capacity (TIBC) were determined by Synlab Czech s.r.o. (Prague, Czech Republic), by colorimetric assay on an AU5822 chemistry analyzer (Beckman Coulter, Olympus, Prague, Czech Republic). Per cent transferrin saturation by iron (TSAT%) was calculated according to the following formula:

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TSAT\% = \left( \frac{\text{plasma iron}}{\text{TIBC}} \right) \times 100
\]

Statistical analysis
Summary statistics were calculated for each variable. Quantitative variables were expressed as mean±standard deviation. One-way analysis of variance or Mann-Whitney rank-sum test was used to compare the variables between the two groups on SD 2, SD 18 and SD 31. Linear regression was analysed for the bodyweight gain from SD 1 to SD 29 and the Hb values at the end of the study (SD 31) (Statgraphics Centurion, V. XVII, Umex, Germany). A P value <0.05 was considered as statistically significant.

RESULTS
No treatment-related adverse events that required veterinary intervention were observed during the study. Two animals from the Forceris group showed a slight temporary swelling at the injection site within the first day of observation after treatment. The bodyweights were not significantly different between the groups on SD 1, the day of randomisation (P=0.53) and the mean bodyweight gains from SD 1 to SD 29 were not significantly different between groups.

On the first day of analysis (SD 2), blood from some animals clotted and could not be used for analysis except iron. Overall, 10 samples from the Forceris group and seven from the Uniferon group could be analysed. On SD 18, 12 samples from the Forceris group and 11 samples from the Uniferon group and on SD 31 samples from all piglets were available for analysis. For the iron parameters (plasma iron, TIBC and TSAT), all samples could be analysed on all sampling days.

For the samples that could be analysed on SD 2, values were comparable between the two groups with some slightly higher values in the Uniferon group (P>0.05; table 1 and figure 1), and 43 per cent–50 per cent of the piglets were borderline anaemic with Hb levels <9 g/dl.

Following treatment (SD 2), the Hb levels increased in both treatment groups. The mean levels were not
significantly different on SD 18, but in the Uniferon group 2/11 piglets showed Hb levels < 11 g/dl while all piglets in the Forceris group had levels well above this value. On SD 31 the mean Hb level was significantly higher in the Forceris group with levels > 9 g/dl in all piglets, while 2/12 piglets in the Uniferon group were anaemic (Hb < 9 g/dl) at that time point (table 1 and figure 1).

When the bodyweight gain of the piglets from SD 1 to SD 29 was compared with the Hb values on SD 31, regression analysis revealed a negative correlation (R²=0.2034; P=0.0236). Separate analysis of the two groups showed this correlation for the Uniferon group (R²=0.3446; P=0.0448) but not the Forceris group (R²=0.0025, P=0.8720).

No significant differences were found for RBC, Ht, MCV, MCHC and CHCM on SD 18, while plasma iron and TSAT% were significantly higher in the Forceris group. By SD 31, Hb, Ht, MCV, MCH, CHCM, plasma iron and TSAT were significantly higher and TIBC was significantly lower in the Forceris group (table 1).

**DISCUSSION**

Iron is an essential component of every cell and tissue in the body with critical roles in transport and storage of oxygen and as part of a large variety of enzymes. It is necessary for cellular growth, proliferation and proper...

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**Table 1** Changes in haematological parameters, plasma iron, total iron binding capacity and transferrin saturation following single intramuscular application of 200 mg iron as Forceris or Uniferon 200

| Parameter | Study day | Mean (sd) | Forceris | Uniferon 200 | P values |
|-----------|-----------|-----------|----------|--------------|----------|
| Red blood cell count (x10^6/µl) | 2 | 4.43 (0.74) | 4.63 (0.99) | NS (P=0.64) |
| | 18 | 5.83 (0.43) | 5.73 (0.43) | NS (P=0.58) |
| | 31 | 6.18 (0.45) | 5.89 (0.30) | NS (P=0.08) |
| Haemoglobin (g/dl) | 2 | 8.88 (1.60) | 9.41 (2.32) | NS (P=0.58) |
| | 18 | 12.23 (0.51) | 11.97 (0.88) | NS (P=0.39) |
| | 31 | 11.30 (0.83) | 10.05 (0.91) | **(P=0.002)** |
| Haematocrit (%) | 2 | 27.60 (4.87) | 28.67 (6.12) | NS (P=0.69) |
| | 18 | 38.76 (2.11) | 38.30 (2.87) | NS (P=0.66) |
| | 31 | 35.60 (2.56) | 32.18 (2.47) | **(P=0.0025)** |
| Mean corpuscular volume (fl) | 2 | 62.27 (1.99) | 62.10 (1.40) | NS (P=0.85) |
| | 18 | 66.66 (4.00) | 66.96 (4.08) | NS (P=0.86) |
| | 31 | 57.8 (1.96) | 54.59 (2.72) | **(P=0.0025)** |
| Mean corpuscular haemoglobin (pg) | 2 | 20.01 (0.85) | 20.28 (1.38) | NS (P=0.61) |
| | 18 | 21.06 (1.04) | 20.92 (1.13) | NS (P=0.77) |
| | 31 | 18.35 (0.70) | 17.06 (1.20) | **(P=0.003)** |
| Mean cell haemoglobin concentration (g/dl) | 2 | 32.14 (1.28) | 32.67 (2.54) | NS (P=0.57) |
| | 18 | 31.59 (0.93) | 31.26 (0.48) | NS (P=0.3091) |
| | 31 | 31.76 (0.58) | 31.22 (0.99) | NS (P=0.1096) |
| Mean corpuscular haemoglobin concentration (g/dl) | 2 | 29.79 (0.74) | 29.70 (0.60) | NS (P=0.7938) |
| | 18 | 29.48 (0.86) | 29.06 (0.56) | NS (P=0.1865) |
| | 31 | 29.81 (0.69) | 29.03 (0.91) | **(P=0.00243)** |
| Iron (µmol/l) | 2 | 14.28 (4.87) | 13.04 (4.61) | NS (P=0.4167) |
| | 18 | 27.77 (4.76) | 18.48 (6.76) | **(P=0.0017)** |
| | 31 | 10.54 (5.17) | 6.57 (2.01) | *(P=0.036)** |
| Total iron binding capacity (µmol/l) | 2 | 51.83 (13.83) | 48.30 (9.42) | NS (P=0.9077) |
| | 18 | 79.69 (10.59) | 92.42 (29.77) | NS (P=0.3539) |
| | 31 | 66.54 (10.15) | 79.18 (16.97) | *(P=0.0127)** |
| Transferrin saturation (%) | 2 | 29.52 (13.53) | 28.37 (13.80) | NS (P=0.9077) |
| | 18 | 35.48 (7.73) | 22.03 (10.30) | **(P=0.0021)** |
| | 31 | 15.91 (7.88) | 9.18 (4.98) | *(P=0.027)** |

For samples sizes, see ‘Materials and methods’. *A significant difference between groups (in bold): *P<0.05, **P<0.01. NS, not significant.
function of the immune system. Pigs kept under intensive conditions lack access to soil, a rich source of iron, and intramuscular iron injection during the first three days of life is considered as the most satisfactory and routine method of prevention of IDA in piglets. However, iron requirements might even be higher under current piglet production conditions, possibly requiring additional dosing during the suckling period. The main reason for the increased demand for iron supplementation is the high fertility of current breeding lines (resulting in large litters), lower average birth weight with large variations within litter in combination with the high growth performance.

Iron deficiency is defined as a reduction in total body iron to an extent that iron stores are fully exhausted and some degree of tissue iron deficiency is present. The deficiency can be mild, without anaemia, or more advanced, resulting in IDA. Poor haematinic activity and IDA are characterised by low Hb levels, low plasma iron, low plasma ferritin, low TSAT and high TIBC. Haemoglobin is the most widely used parameter for the detection of anaemia and for the evaluation of the biological response to iron supplementation. However, Hb values in sucking piglets are highly variable, and the physiological levels, and consequently anaemia, are defined differently. A Hb value of 9 g/dl is most frequently considered as the cut-off for anaemia. In this study, values of 9–11 g/dl were considered as normal or first-stage anaemia, Hb <9 g/dl as second-stage anaemia.

Plasma iron, TIBC and TSAT may also serve as informative markers of the iron status. Plasma iron represents the iron bound to transferrin, which is available to be incorporated into Hb. TIBC indicates the amount of iron that plasma could bind and is increased in anaemic piglets. Plasma iron in combination with TIBC as well as TSAT% and ferritin are considered as haematological indices for improved early indicators of anaemia. Plasma ferritin alone or in combination with TIBC is directly proportional to the body iron store in healthy individuals.

Following intramuscular administration of 200 mg of iron at birth, Hb levels might be depleted as early as 17 days of age, especially in fast-growing piglets. Therefore, evaluation of Hb levels during this period can provide additional, more detailed information about the iron status and potential iron gaps. While the mean Hb levels were still comparable between the two groups on SD 18, two animals in the Uniferon group already showed levels <11 g/dl (first-stage iron deficiency) at that time point. A significant difference in the mean Hb values was observed on the last day of sampling (SD 31) and 2 out of 12 piglets in the Uniferon group had levels <9 g/dl indicating second-stage iron deficiency, and only 2 with levels >11 g/dl in the Uniferon group, suggesting a more effective haematinic activity of gleptoferron.

It is frequently discussed that especially large, fast-growing piglets are at risk of development of first-stage or second-stage anaemia, and in the present study Hb values were negatively correlated with bodyweight gain by the end of the observation period. When the two groups were analysed separately, this correlation was only seen in the Uniferon but not in the Forceris group. One of the reasons for differences between the tested products may be a difference in the total amount of absorbed iron. It could be shown that gleptoferron has a 4.6 times higher total iron absorption compared with iron dextran, and this resulted in a more sustainable iron supply until the end of the study.

Compared with Hb, the interpretation of plasma iron biochemistry in piglets is not as straightforward since reference values differ. However, a relative change in serum iron concentration and %TSAT which decline together with the rise of TIBC during iron deficiency can be considered as more sensitive early indicators than Hb evaluation alone.

For the other haematological parameters (RBC, Ht, MCV, MCHC, CHCM), there were no significant
differences on SD 18. On SD 31, Ht, MCV, MCH and CHCM were significantly higher in the Forceris group but still in the physiological range as suggested. In the Uniferon 200 group, except for the mean MCV (SD 31). While classical erythrocyte indices like RBC usually do not provide information on rapid changes in erythropoietic activity due to the long erythrocyte life-span, MCV and MCH values together with reticulocyte numbers have been described as reliable parameters for early iron deficiency, reflecting recent bone marrow activity. A reduction in MCV can usually be expected when iron deficiency persists for several weeks and microcytic cells are produced in increased numbers. In the present study, a significant reduction of MCV on SD 31 supports the hypothesis that availability of iron applied once as iron dextran is reduced in older piglets compared with gleptoferron.

The present study evaluated the application of gleptoferron in combination with the anticoagulid toltrazuril in an injection formulation designed to reduce the handling and therefore stressful events of newborn piglets that require both iron supplementation and metaphylaxis against C. suis. A similar combination for oral application was developed recently and marketed in South America. Oral iron administration provided lower Hb levels on SD 21 in comparison with the iron injected control group (9.87 g/dl vs 11.53 g/dl) which can be considered as a subclinical anaemia status. Limited efficacy of dietary iron supplementation was reported previously and is presumed to be due to the immature duodenal iron absorption physiology of sucking piglets. An injectable formulation including toltrazuril and iron is therefore considered advantageous for iron supplementation with comparable efficacy of toltrazuril compared with the oral formulation.

CONCLUSION
The results of this comparative study provide insight into the efficacy of Forceris, a new combination product designed to control both coccidiosis and IDA in piglets. The tested products were efficient in preventing IDA. Forceris demonstrated a better anti-anaemic activity compared with Uniferon with no anaemic piglets and higher plasma iron and haematological performances at the end of the study.

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Contributors AJ, HK and DS designed the study and drafted the manuscript AS and BF analysed the samples and supervised the animal study part HK carried out the statistical analysis and BH carried out the dispensing, blinding and deblinding of the staff involved and the sponsor. All authors have read and approved of the submitted version of the manuscript.

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Competing interests DS and HK are employees of Ceva Santé Animale. No member of the staff of the Vetmeduni Vienna involved in the trial received allowances or other personal benefits from the sponsor.

Ethics approval The procedures involving piglets were approved by the institutional ethics committee and the national authority according to § 26ff of Animal Experiments Act, Tierversuchsgesetz 2012 – TVG 2012 (licence number: BMWF-68.205/0034-WF/3b/2016; Austrian Federal Ministry of Science, Health and Economy).

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