Development of a Competitive ELISA for Detection of *Leptospira borgspeterseni* serovar hardjo in Vaccinated Cattle

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Introduction

There are many reports on the use of ELISAs as tests for the detection of leptospiral antibodies, however, no test to date has been developed that has the potential to discriminate between vaccinated and naturally infected animals.

Materials and Methods

A DNA insert which encoded for a section of protein of serovar hardjo (type bovis) was cloned into a pTrcHis vector and then overexpressed in *E. coli*. The resulting recombinant fusion protein (JMC) was purified by metal affinity chromatography. Expression of the protein was confirmed by western blotting with anti-JMC specific monoclonal antibodies (Mabs). A competitive ELISA (cELISA) was developed for serological testing of sera, the recombinant protein JMC was used as antigen and anti-JMC Mabs to increase the specificity of the ELISA.

Results and Conclusion

The cELISA detected cattle vaccinated with a type bovis vaccine, but not cattle vaccinated with a type prajitno vaccine. Naturally and experimentally infected cattle showed no significant reaction to the JMC protein.

Effect on *in-vitro* Cell Growth of Various Rehydrant Solutions

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Introduction

In a controlled clinical study, the addition of Psyllium (a mucopolysaccharide derived from Isphagula seed shells) to a standard isotonic oral rehydrant solution (Deliver® with Dialine®, Pharmalett A/S, Denmark) reduced the duration, frequency and severity of diarrhea in newborn calves. This improvement of treatment is believed to be due to prolonged transit time through the small intestine, resulting in increased absorption of glucose and electrolytes.

A new isotonic rehydrant solution (Deliver Extra, Pharmalett A/S, Denmark) was evaluated for its effect on *in vitro* cell growth. Deliver Extra is identical to Deliver® with Dialine® plus a mixture of important amino acids, including extra glutamine. Glutamine is included in Deliver Extra because it is a "limiting" amino acid that may play a role in diarrheal disease. It plays a role in maintaining mucosal integrity of the gut, and in controlled situations, glutamine has also been successful in enhancing the recovery of the damaged intestinal mucosa.

Materials and Methods

In order to investigate cell growth, seven different cell culture media were made. To quantify cell growth, the number of living cells was estimated by measuring lactase dehydrogenase (LDH) activity. LDH activity was measured on day 0, day 1, day 3, day 7 and day 14.

The cells used were provided from human foreskins obtained from the surgical department of Academic Medical Center, Amsterdam, The Netherlands. The following culture medium solutions were made and tested:

Solution I contained a standard culture medium, Dulbecco's Modified Eagle's Medium (DMEM) and fetal calf serum (including glutamine). This was the positive test solution.

Solution II contained solution I and Psyllium.

Solution III contained DMEM and Deliver Extra.

Solution IV contained DMEM and Deliver® with Dialine® (isotonic rehydrant including Psyllium).

Solution V contained DMEM and a standard isotonic WHO rehydrant.

Solution VI contained DMEM and a commercial available hypertonic rehydrant.

Solution VII contained DMEM and solution V and a mixture of important amino acids, including extra glutamine.

Results and Discussion

The optimal effect on cell growth was, as expected, achieved by fetal calf serum with or without Psyllium (solutions I and II). However, the addition of Psyllium to the positive test solution resulted in an extra 25% LDH activity on days 3 and 7. On day 14, the LDH activity of the two solutions was the same; a 5-fold increase compared to day 0.

The two isotonic rehydrant solutions (Solution IV and V) did not influence the activity of LDH during the test period of 14 days, i.e., there was neither a negative nor a positive effect on cell proliferation. Addition of glutamine to a standard isotonic WHO rehydrant (solution VII) doubled the LDH activity on day 14 compared to a standard WHO rehydrant without glutamine (solution V). The addition of a mixture of amino acids, including extra glutamine, to a commercially available isotonic rehydrant formulation (Deliver® with Dialine®; solution III) improved the effect of growth media on cell proliferation 3-fold compared to Deliver® with Dialine® (solution IV). The effect on cell growth by Deliver Extra (solution III) was approximately 50% better than the standard WHO rehydrant that included a mixture of important amino acids plus extra glutamine, and was about 60% of the LDH activity for the positive control media. A hypertonic rehydrant (solution VI) had an instant negative effect on cell growth. On day 3, no LDH activity was measured (i.e., all cells were dead).

Conclusion

Addition of a mixture of amino acids, including extra glutamine, to commercially available isotonic rehydrant solutions improved cell proliferation by 3-fold. Addition of Psyllium improved cell growth even further. On day 14, cell proliferation was about 60% of that of fetal calf serum standard medium. Isotonic rehydrant solutions did not influence cell growth at all. A hypertonic rehydrant solution killed the cells within 3 days.