Sero-epidemiological study of peste des petits ruminants in sheep and goats in India between 2003 and 2009

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Summary
This study describes the serosurveillance of peste des petits ruminants (PPR) in sheep and goats that was carried out between 2003 and 2009 using serum samples from animals suspected of PPR that were submitted to the Rinderpest and Allied Disease Laboratory (Division of Virology of the Indian Veterinary Research Institute [IVRI]). A total of 2,197 serum samples from sheep and 2,687 from goats were screened for PPR virus (PPRV) antibody using a monoclonal antibody-based competitive enzyme-linked immunosorbent assay developed at IVRI. Screening of the 4,884 serum samples showed that the prevalence of PPRV antibody in sheep and goats was 41.01% (95% confidence interval [CI]: 31.86 to 50.16) and 46.11% (95% CI: 37.18 to 55.04), respectively, with an overall prevalence of 43.56% (95% CI: 36.78 to 50.34) during the period. This indicates increased and widespread infection with the virus in India compared with earlier reports, which is attributed to the variations in sheep and goat husbandry practices in different regions, the agro-climatic conditions, the topography of different states, the socio-economic status of individual farmers and the migration of livestock in India.

Keywords
Goat – India – Peste des petits ruminants – Serosurveillance – Sheep.

Introduction
Peste des petits ruminants (PPR) is an acute, highly contagious, notifiable and economically important transboundary viral disease of goats and sheep, which is listed by the World Organisation for Animal Health (OIE). The mortality usually ranges from 50% to 90%, although it sometimes can be zero, and morbidity varies from 10% to 100%, or sometimes lower than 10%, depending on circumstances (2). The disease is considered to be one of the main constraints to improving the productivity of small ruminants in enzootic countries (14). The causative agent, PPR virus (PPRV), belongs to the Morbillivirus genus of the Paramyxoviridae family. It affects sheep and goats primarily, and occasionally infects wildlife. The disease is characterised clinically by severe pyrexia, ocular nasal discharge, necrotising and erosive stomatitis, enteritis and pneumonia (10, 18, 31). Although PPRV has a single serotype, it is grouped genetically into four lineages (I, II, III and IV) on the basis of partial sequence analysis of the
fusión (F) gene. Lineages I to III circulate in Africa and lineage IV in Asia (6, 22). Peste des petits ruminants was first reported in Côte d’Ivoire in West Africa (10), and later in other parts of the world, namely sub-Saharan Africa, the Arabian Peninsula, the Middle East and the Indian subcontinent (22). In recent years, the disease has also been reported from China (34) and Morocco (35), which raises the threat of its introduction into Europe.

In India, PPR was first recorded in 1987 from Arasur village, in the Villupuram district of Tamil Nadu (21), and it continued to be present in the southern peninsula until 1994. Later, a number of PPR outbreaks were reported from the northern states of India (13, 16), with a solitary report in Indian buffalo in a southern state (11). Peste des petits ruminants is enzootic in India, and outbreaks occur in small ruminants, such as sheep and goats, regularly throughout the country (13, 24). It is a major constraint on small ruminant production (24), causing great economic losses because of morbidity, mortality, and losses of productivity due to trade restrictions. Economic losses due to PPR have been estimated to be 1,800 million INR (US$39 million) annually (23, 33).

Small ruminants are important livestock species, both numerically and economically, in developing countries such as India. The world population of sheep and goats is approximately 2.1 billion, of which India has about 78 million sheep and 140 million goats (5). In India, small ruminants make important contributions to the lives of small, marginal and landless rural farmers and allow them to sustain their livelihood by providing meat, fibre, milk, skin and manure (20). The husbandry of small ruminants also generates self-employment, raises income, improves household nutrition and plays an important role in sustainable agriculture and generation of employment (3, 12). The proportion of sheep to goats and the population density vary greatly under different agro-climatic conditions.

Information on the prevalence of antibodies to PPRV in small ruminants and other species is available from a number of countries in which the disease is reported, including the Sultanate of Oman, Jordan, Sudan, Turkey and various African countries (1, 8, 17, 30). Only a few reports have described systematic study of the pattern of PPRV infection and its seroprevalence in small ruminants in India (19, 24). The prevalence of PPRV antibodies in sheep and goats indicates subclinical or inapparent or non-lethal clinical infection, which may be of epidemiological significance. Efficient and sensitive diagnostic tests are a great help in rapidly providing evidence that PPRV is not circulating in a free-ranging population. Data on the molecular epidemiology and sero-epidemiology of the disease play an important role in effective disease management. A monoclonal antibody (MAb)-based competitive enzyme-linked immunosorbent assay (c-ELISA) and a sandwich ELISA, for detection of PPRV antibody and antigen respectively, were developed at the Indian Veterinary Research Institute (IVRI), Mukteswar (25, 26). These are the tests currently employed for serosurveillance and seromonitoring of the clinical prevalence of PPR throughout India.

In view of the economic importance of the disease and the dense sheep and goat population in the region, the authors undertook the present study with the objectives of generating baseline data on the prevalence of PPR in India between 2003 and 2009, and of investigating the sero-epidemiology of the disease using serum samples from sheep and goats suspected of the disease.

Materials and methods

Clinical samples
Between 2003 and 2009 a total of 4,884 serum samples of unknown antibody status (n = 2,197 [sheep]; n = 2,687 [goats]) were submitted to the Rinderpest and Allied Disease Laboratory (Division of Virology, IVRI) for diagnosis of PPR. The samples came from different geographical locations in India from various sources, including intensive sheep/goat farms, small farms, field investigations, state animal husbandry laboratories, and research institutes. The samples originated from both intensive and extensive husbandry sectors. The majority of serum samples were received from the northern and central parts of the country. None of the animals from which the samples were collected had a history of PPR vaccination. The details of the species from which the samples were collected and the area of origin are presented in Table I. All the clinical samples were stored at −20°C upon receipt from the field and were used for further analysis when required.

Competitive enzyme-linked immunosorbent assay
A PPR c-ELISA kit (26) developed at IVRI was used for detection of antibodies against PPRV, which were measured in terms of percentage inhibition (PI). The serum samples were tested according to the protocol described by Singh et al. (26). Samples with PI of ≥50% were considered positive for the presence of PPRV antibodies.

Statistical analysis
The estimation of apparent prevalence with 95% confidence intervals (CI) and the chi-squared test (χ²) were carried out using the data obtained in the study (27). The
apparent prevalence and true prevalence were also estimated from the following formula (32):

Apparent prevalence = number of positive animals/number of tested animals

True prevalence = (apparent prevalence + [specificity − 1]) / (sensitivity + specificity − 1)

The true prevalence was calculated on the basis of the sensitivity and specificity of the c-ELISA employed in the study, which has high relative specificity (98.4%) and sensitivity (92.4%) when compared with the virus neutralisation assay (26). Further, seroprevalence rates were depicted on the regional map of India using the EpiInfo™ software-2000 designed by the Centers for Disease Control and Prevention in the United States.

Results and discussion

Peste des petits ruminants has great potential to cause high economic losses to the livestock industry in Africa, the Middle East and Asia. For the effective control and eradication of PPR, strong support of diagnostics and timely vaccination of the susceptible population, based on an understanding of the epidemiology of the disease, are imperative. Eradication of the disease depends on rapid and accurate diagnosis of infection and the implementation of prompt control measures. Peste des petits ruminants played a role in the control of rinderpest, and might also have helped to eradicate the disease, because seroconversion of cattle with cross-reacting PPRV antibodies is possible where small and large ruminants co-exist (31). In India, owing to inadequate animal disease reporting and surveillance systems, several PPR outbreaks have not been recorded properly. Measurement of the prevalence of antibodies to PPRV in different geographical areas of the country with varying agro-climatic conditions may be helpful in developing disease control strategies. Organised serological surveys of the nationwide prevalence of PPR in India have rarely been conducted. The majority of reports have described only regional data, with only a few exceptions (19, 24). A small number of reports published since 1994, from various states of India, have generally indicated that most positive animals have migrated from neighbouring states (29).

The present investigation provided preliminary data on PPRV infection in India between 2003 and 2009 in sheep and goats suspected of the disease. Based on the screening of the 4,884 serum samples, the apparent seroprevalence was 41% and 46% in sheep and goats, respectively, with an overall prevalence of 43.56% (Table I). A higher seroprevalence was observed in goats than in sheep in this study. This may be because the samples were collected from suspected cases of PPR, although there was no significant difference observed in the prevalence of clinical disease between sheep and goats. Soundararajan et al. (28) reported a higher mortality in infected goats than in sheep. That study was conducted on a large intensive farm that was probably suffering an outbreak, a situation similar to that of the field samples received from animals suspected

<table>
<thead>
<tr>
<th>State</th>
<th>Sheep Tested</th>
<th>Sheep Number positive</th>
<th>Sheep Percentage positive</th>
<th>Goats Tested</th>
<th>Goats Number positive</th>
<th>Goats Percentage positive</th>
<th>Cumulative total</th>
<th>Cumulative positive</th>
<th>Cumulative positive (%)</th>
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<td>10</td>
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<td>0</td>
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<td>0.00</td>
<td>119</td>
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<td>Gujarat</td>
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<td>18.85</td>
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<td>167</td>
<td>42.27</td>
<td>623</td>
<td>210</td>
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<td>48.89</td>
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<td>Himachal Pradesh</td>
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<td>8</td>
<td>9.19</td>
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<td>57</td>
<td>24.78</td>
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<td>63</td>
<td>48.84</td>
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<td>103</td>
<td>51.24</td>
<td>330</td>
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<td>50.30</td>
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<td>29.15</td>
<td>40</td>
<td>26</td>
<td>65.00</td>
<td>239</td>
<td>84</td>
<td>35.15</td>
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<td>616</td>
<td>365</td>
<td>59.25</td>
<td>575</td>
<td>336</td>
<td>58.43</td>
<td>1191</td>
<td>701</td>
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<tr>
<td>Rajasthan</td>
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<td>252</td>
<td>40.00</td>
<td>205</td>
<td>132</td>
<td>64.39</td>
<td>835</td>
<td>384</td>
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<tr>
<td>Tripura</td>
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<td>13</td>
<td>100.00</td>
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<td>100.00</td>
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<td>Uttar Pradesh*</td>
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<td>102</td>
<td>34.23</td>
<td>859</td>
<td>379</td>
<td>44.12</td>
<td>1157</td>
<td>481</td>
<td>41.57</td>
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<tr>
<td>West Bengal</td>
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<td>0</td>
<td>0.00</td>
<td>5</td>
<td>4</td>
<td>80.00</td>
<td>5</td>
<td>4</td>
<td>80.00</td>
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<tr>
<td>Total</td>
<td>2,197</td>
<td>901</td>
<td>41.01 ± 8.86</td>
<td>2,687</td>
<td>1,239</td>
<td>46.11 ± 8.53</td>
<td>4,884</td>
<td>2,140</td>
<td>43.56 ± 8.66</td>
</tr>
</tbody>
</table>

CI: 95% confidence interval
*The origin of some of the samples submitted by the Centre for Animal Disease Research and Diagnosis in Uttar Pradesh was not known, but they have still been included in the figures for this state

Table I
Seroprevalence of peste des petits ruminants in sheep and goats by state for the 2003–2009 period
of PPR in this study. This could be the major reason for the higher seropositivity in goats than in sheep in this study; other serological studies of PPRV have not shown the same results (24). For example, Singh et al. (24) recorded an average seropositivity of 33%, with a higher prevalence of antibodies to PPRV in sheep (36.3%) than in goats (32.4%), using random serum samples obtained mostly from northern India.

The high seroprevalence may explain the difficulties experienced in achieving high post-vaccination levels of immunity against PPR (24). A recent study indicated a higher prevalence of antibodies in sheep (41.35%) than in goats (36.91%) when screening random samples collected from the field (19). In contrast, the present study found an overall seroprevalence of PPR in sheep and goats suspected of infection of 43.56%, with a higher prevalence in goats than in sheep. These results demonstrate the widespread nature of the disease in India more directly than earlier observations that involved the testing of random samples (19, 24). Chavan et al. (4) reported an overall seroprevalence of PPR in goats of 46.01%, with a range of 42.30% to 52.94%, at different locations in the Parbhani region of Maharashtra.

In most previous reports, goats were affected more severely with the disease than sheep. Sheep might have greater innate resistance to clinical development of the disease than goats; this assumption is supported by observations in several outbreaks of PPR in India, as reported previously (24). It is also possible that the virus selectively infects goats rather than sheep when both the natural hosts are reared contiguously. However, the sheep-adapted virus shows equal severity in sheep in areas of intensive husbandry where only sheep are available for infection (24). In India, sheep and goats are reared mostly by nomads and are maintained in a free-range pattern or grazing on pasture. They receive minimal veterinary care, which may increase the chance of the animals acquiring infection. When the lean period starts these animals migrate in search of pasture and are also transported for trade purposes (24).

The prevalence values reported here are in concordance with reports from other countries. In Africa, PPRV antibodies were reported in 55% of Nigerian sheep and goats (15), and in 46.5% of sheep and goats from Cameroon (8). Studies of the seroprevalence of PPR in sheep and goats have also been reported from Sudan, Saudi Arabia and Ethiopia (1, 17). In a recent study from Tanzania, significantly more seropositive individuals were found among goats than among sheep (49.5% versus 39.8%, \( p = 0.02 \)), with an overall seroprevalence of 45.8% reported (30). Variations in seroprevalence could be due to differences in sample size, age, prevailing management practices, humidity or season (24). The susceptibility of a host to infection with PPRV varies with the breed of the animal, which also plays an important role in the epidemiology of PPR (7, 9, 14). In this study, the susceptibility of different sheep and goat breeds could not be assessed as no proper breed information was supplied.

Serum samples screened for PPRV antibody and their percentage seropositivity, with the prevalence, are presented for each state in Table I and by year in Table II; they are also depicted on a map and in a graph (Figs 1a and 1b, Fig. 2). In this study, no statistically significant difference was observed in sheep or goats between 2003 and 2009. The seroprevalence of PPR was high during 2005–2006 and low during 2003–2004 and 2004–2005 in both sheep and goats (dates refer to 12-month periods running from April to March). The increase in the number of positive samples between 2003 and 2006 is interesting (Table II). This finding may be related to the increase in the incidence of the disease from 1995 onwards (16); the disease is now enzootic in northern India, as reported.

### Table II

<table>
<thead>
<tr>
<th>Year*</th>
<th>Sheep</th>
<th>Goat</th>
<th>Sheep</th>
<th>Goat</th>
<th>Apparent</th>
<th>True</th>
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<tbody>
<tr>
<td>(CI: 31.86 to 50.16)</td>
<td>(CI: 37.18 to 55.04)</td>
<td>(CI: 36.78 to 50.34)</td>
<td>(CI: 35.72 to 51.82)</td>
<td>(CI: 41.51 to 57.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003–2004</td>
<td>567</td>
<td>169</td>
<td>29.81</td>
<td>456</td>
<td>187</td>
<td>41.01</td>
</tr>
<tr>
<td>2004–2005</td>
<td>253</td>
<td>112</td>
<td>44.27</td>
<td>484</td>
<td>166</td>
<td>34.30</td>
</tr>
<tr>
<td>2005–2006</td>
<td>580</td>
<td>323</td>
<td>55.69</td>
<td>610</td>
<td>334</td>
<td>54.75</td>
</tr>
<tr>
<td>2006–2007</td>
<td>292</td>
<td>114</td>
<td>39.04</td>
<td>630</td>
<td>276</td>
<td>43.81</td>
</tr>
<tr>
<td>2007–2008</td>
<td>256</td>
<td>101</td>
<td>39.45</td>
<td>184</td>
<td>87</td>
<td>47.28</td>
</tr>
<tr>
<td>2008–2009</td>
<td>249</td>
<td>82</td>
<td>32.93</td>
<td>323</td>
<td>189</td>
<td>58.51</td>
</tr>
<tr>
<td>Total</td>
<td>2,197</td>
<td>901</td>
<td>41.01 ± 3.74</td>
<td>2,687</td>
<td>1,239</td>
<td>46.11 ± 3.65</td>
</tr>
</tbody>
</table>

*12-month period running from April to March
CI: 95% confidence interval
previously (24). However, the extensive application of live attenuated PPR vaccines in the field and the supply of diagnostic kits to field laboratories in different states within the country (23) have led to a change in the disease pattern in the recent past. This has been demonstrated by a decrease in the number of outbreaks in the field and a decrease in the number of samples received for diagnosis at the Rinderpest and Allied Disease Laboratory (23). No statistically significant differences among the various states of India in the prevalence of PPR antibodies in sheep and goats were observed. The 95% CI for the seroprevalence in sheep and goats in all the states combined was 23.65–58.37 and 29.39–62.83, respectively. The seroprevalence was low in the state of Himachal Pradesh, in both sheep and goats, which agrees with previous reports (24). This may be due to the topology of the region, which causes restricted movement of animals, with reduced transmission of the virus between animals. Sheep in Maharashtra and goats in Rajasthan showed a high seroprevalence of PPR. The overall prevalence was highest in Maharashtra (58.86%), followed by Jammu and Kashmir (50.30%) and Rajasthan (45.99%), when compared with other states of India on the basis of the number of samples received for analysis. The regional difference in the prevalence of antibodies is based on the relative populations of sheep and goats. A relatively high proportion (70–80%) of goats means that the population is at risk of infection, particularly in the northern parts of the country, as reported earlier (24). Few serum samples were received for serodiagnosis from Andhra Pradesh, Tripura, Assam, Haryana and West Bengal, which suggests a lower prevalence of PPR than in other states, even though all the samples submitted from these states were positive for PPRV antibodies. A prevalence of PPRV antibodies ranging between 0% and 2.1% was reported previously for the north-eastern Indian states of Assam and Meghalaya (24). These states are geographically isolated and have a relatively small population of sheep and goats. However, transboundary migration of animals must be monitored for proper management of disease, especially in the border states of India.

The implementation of a national PPR control programme would probably change the epidemiology of PPR in India.
in terms of the distribution of the disease. It is hoped that PPR, in the same way as rinderpest, will be eradicated in India within a decade. This goal can be achieved by employing comprehensive active intensive surveillance programmes in enzootic areas and then implementing intensive vaccination campaigns in these areas. A vaccination programme would need to take into account the population dynamics of goats and sheep in India. The present survey provides only preliminary information on PPR sero-epidemiology, because the samples analysed may not be a true representation of the target population. However, the information will be very useful in the formulation of effective disease management strategies and in the implementation of a PPR control programme. More systematic, intensive and comprehensive active serological surveillance, along with measurement of clinical prevalence in the enzootic parts of the country, must be undertaken in order to develop effective control strategies for PPR. Active serosurveillance is only possible in collaboration with the state animal husbandry departments of the respective regions. Efforts are already being made to generate more active surveillance data on the epidemiology of PPR in livestock species, including wild ruminants, in southern peninsular India through collaboration with the Project Directorate on Animal Disease Monitoring and Surveillance in Bangalore.

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Étude séro-épidémiologique de la peste des petits ruminants chez les ovins et caprins en Inde entre 2003 et 2009


Résumé

Les auteurs décrivent les résultats d’une enquête sérologique relative à la peste des petits ruminants (PPR), conduite entre 2003 et 2009 en Inde. L’étude s’est basée sur les échantillons sériques issus d’animaux suspects de PPR qui avaient été adressés pour analyse au Service de virologie de l’Institut indien de recherche vétérinaire (IVRI). Au total, 2 197 sérums ovins et 2 687 sérums caprins ont été soumis à l’épreuve immuno-enzymatique de compétition recourant à un anticorps monoclonal, épreuve mise au point par l’IVRI pour détecter la présence d’anticorps dirigés contre le virus de la PPR. L’analyse des 4 884 échantillons sériques a montré que les taux de prévalence des anticorps dirigés contre le virus de la PPR s’élevaient respectivement à 41,01 % chez les ovins (intervalle de confiance [IC] à 95 % compris entre 31,86 et 50,16) et à 46,11 % chez les caprins (IC à 95 % compris entre 37,18 et 55,04), avec une prévalence totale de 43,56 % pour la période considérée (IC à 95 % compris entre 36,78 et 50,34). Ces résultats indiquent que l’intensité et l’extension de l’infection virale ont fortement augmenté en Inde depuis les précédents rapports, ce que les auteurs attribuent aux modifications des pratiques de l’élevage ovin et caprin dans les différentes régions, aux variations des conditions agro-climatiques, à la diversité de la topographie selon les états, à la situation socio-économique dans laquelle se trouvent les éleveurs et aux déplacements de bétail en Inde.

Mots-clés

Chèvre – Inde – Mouton – Peste des petits ruminants – Séro-surveillance.
Estudio seroepidemiológico de la peste de pequeños rumiantes en caprinos y ovinos de la India entre 2003 y 2009


Resumen
Los autores describen los resultados de un proceso de serovigilancia de la peste de pequeños rumiantes (PPR) en ovinos y caprinos instaurado entre 2003 y 2009 en la India. Para ello se utilizaron muestras séricas de animales presuntamente enfermos, que se enviaron a la División de Virología del Instituto de Investigación Veterinaria de la India (IVRI). En total, 2.197 muestras de suero ovino y 2.687 de suero caprino fueron sometidas a un ensayo inmunoenzimático (ELISA) de competición con anticuerpos monoclonales, elaborado en el propio IVRI, para detectar en ellas anticuerpos contra el virus de la PPR. El análisis de las 4.884 muestras puso de manifiesto una prevalencia de anticuerpos contra el virus de la PPR en ovinos y caprinos del 41,01% (intervalo de confianza [IC] del 95%: 31,86 a 50,16) y del 46,11% (IC 95%: 37,18 a 55,04), respectivamente, y una prevalencia total del 43,56% durante el periodo de estudio (IC 95%: 36,78 a 50,34). Tales resultados ponen de relieve que la intensidad y extensión de la infección por el virus se han acrecentado en la India en relación con los datos de estudios anteriores, hecho que se atribuye a la evolución de las técnicas de producción ovina y caprina en las diferentes regiones, a las condiciones agroclimáticas, a la topografía de los distintos estados, a la situación socioeconómica de los productores y al desplazamiento de rebaños en la India.

Palabras clave
Caprino – India – Ovino – Peste de pequeños rumiantes – Serovigilancia.

References


